NOVEL CHEMICAL COMPOUNDS AND THEIR USE

The present invention relates to new chemical compounds and their use in medicine. In particular the invention concerns prodrugs of pharmaceutical moieties, more specifically antimicrobial agents, methods for their preparation, pharmaceutical formulations containing them and their use in the treatment of microbial infections.

BACKGROUND OF THE INVENTION

The use of prodrugs as progenitors of pharmaceutical moieties is widespread and there are numerous examples of prodrug therapeutics that are converted to active drugs *in vivo*. Such prodrugs may be designed to improve absorption of pharmaceutical agents by, for example, the gastrointestinal tract.[1] Prodrugs may also be produced to facilitate transport across the blood-brain barrier,[2] provide slow release of pharmaceutically active agents,[3] improve patient acceptance[4] and minimise side effects.[5]

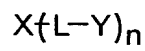
SUMMARY OF THE INVENTION

We have now developed prodrugs of pharmaceutical moieties, more specifically antimicrobial agents. These prodrugs are designed with the specific purpose of increasing residency time at epithelial surfaces such as the lung and airways, urinary and gastrointestinal tracts, blood vessels and skin. To accomplish this, the pharmaceutical moieties are converted to prodrugs by modification with a pharmacokinetic regulator by way of a linker group that can be cleaved *in vivo* to expose the drug.

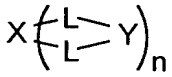
It is envisaged that interaction with cell membranes will also increase residence times in major organs such as the liver and central nervous system given appropriate routes of administration. Organ specific

targeting groups (labile or otherwise) could also be attached to the prodrug to enhance the delivery process.

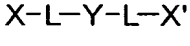
In a first aspect, the present invention provides a prodrug of the general Formula (I), (II) or (III):



(I)



(II)

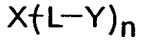


(III)

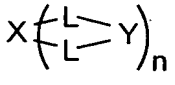
in which

X is a tobramycin moiety;
X' is a pharmaceutically active moiety;
L is a linker group;
Y is a pharmacokinetic regulator; and
n is an integer of 1 or greater
or a pharmaceutically acceptable derivative or salt thereof.

The invention also provides a prodrug of general Formula (I), (II) or (III):



(I)



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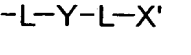


(III)

in which

X and X' are either the same or different and selected from an aminoglycoside excluding tobramycin;

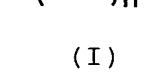
L is a linker group excluding amide and carbamate;

Y is a pharmacokinetic regulator; and

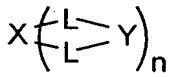
n is an integer of 1 or greater

or a pharmaceutically acceptable derivative or salt thereof.

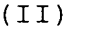
The invention further provides a prodrug of the general Formula (I), (II) or (III):



(I)



(II)



(III)

in which

X and X' are either the same or different and selected from a nucleoside, rhinovirus capsid-binding compound, antisense oligonucleotide, peptide, an inhibitor of HIVRT, an inhibitor of influenza neuraminidase, amphotericin β, an azole and an aspartic proteinase;

L is a linker group;

Y is a pharmacokinetic regulator; and

n is an integer of 1 or greater

or a pharmaceutically acceptable derivative or salt thereof.

In a second aspect, the present invention provides a method for the preparation of the prodrug as defined above which comprises the steps of:

(a) optionally protecting the pharmaceutically active moieties X and/or X' and/or the linker group which is attached to the optionally protected pharmacokinetic regulator Y;

(b) reacting the optionally protected pharmaceutically active moieties X and/or X' and the optionally protected linker group L attached to the optionally protected pharmacokinetic regulator Y; and

(c) if necessary, removing the protecting groups of the pharmaceutically active moieties X and/or X', the linker L and the pharmacokinetic regulator Y.

In a third aspect, the invention provides the prodrug as defined above or a pharmaceutically acceptable derivative thereof, for use as an active therapeutic agent in the prevention and/or treatment of a microbial infection.

A fourth aspect of the invention provides a method for the prevention and/or treatment of a microbial infection comprising the step of administering to a subject in need thereof an effective amount of the prodrug as defined above or a pharmaceutically acceptable salt or derivative thereof.

In a fifth aspect, the invention provides use of the prodrug as defined above for the manufacture of a medicament for the prevention and/or treatment of a microbial infection.

In a sixth aspect, the invention provides a method for the detection of a microbial infection which comprises the step of contacting the prodrug as defined above with a sample suspected of containing the microorganism.

In a seventh aspect, the invention provides a pharmaceutical formulation comprising the prodrug as defined above or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers and, optionally, other therapeutic and/or prophylactic ingredients.

According to an eighth aspect of the present invention there is provided an inhaler which contains the formulation as defined above.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual

publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The pharmaceutically-active moiety X may be selected from synthetic or natural peptides, proteins, mono- or oligosaccharides, sugar-amino acid conjugates, sugar-peptide conjugates, toxins, drugs, pro-drugs or drug like molecules. Also included for moiety X are antibodies or antigen binding fragments of whole antibody, wherein the fragments retain the binding specificity of the whole antibody molecule. The binding fragments include, for example, Fab, F(ab')2, and Fv fragments. Binding fragments can be obtained using conventional techniques, such as proteolytic digestion of antibody by papsin or pepsin, or through standard genetic engineering techniques that are known in the art.

Indeed, the present invention is intended to encompass and be suitable for any pharmaceutically active moiety, especially any of the following drugs:

1. Analgesic anti-inflammatory agents such as, acetaminophen, aspirin, salicylic acid, methyl salicylate, choline salicylate, glycol salicylate, l-menthol, camphor, mefanamic acid, fluphenamic acid, indomethacin, diclofenac, alclofenac, ibuprofen, ketoprofen, naproxene, pranoprofen, fenoprofen, sulindac, fenbufen, clidanac, flurbiprofen, indoprofen, protizidic acid, fentiazac, tolmetin, tiaprofenic acid, bendazac, bufexamac, piroxicam, phenylbutazone, oxyphenbutazone, clofezone, pentazocine, mepirizole and the like;

2. Drugs having an action on the central nervous system, for example sedatives, hypnotics, antianxiety agents, anticholinesterase agents, analgesics and anesthetics, such as, chloral, buprenorphine, naloxone, haloperidol, fluphenazine, pentobarbital, phenobarbital, secobarbital, amobarbital, cydobarbital, codeine, lidocaine, tetracaine, dyclonine, dibucaine, cocaine, procaine, mepivocaine,

bupivacaine, etidocaine, prilocaine, benzocaine, fentanyl, nicotine, galanthamine and the like;

3. Antihistaminics or antiallergic agents such as, diphenhydramine, dimenhydrinate, perphenazine, triprolidine, pyrilamine, chlorcyclizine, promethazine, carbinoxamine, tripelennamine, brompheniramine, hydroxyzine, cyclizine, meclizine, cloprenaline, terfenadine, chlorpheniramine and the like;

4. Acetonide anti-inflammatory agents, such as hydrocortisone, cortisone, dexamethasone, fluocinolone, triamcinolone, medrysone, prednisolone, flurandrenolide, prednisone, halcinonide, methylprednisolone, fludrocortisone, corticosterone, paramethasone, betamethasone, ibuprophen, naproxen, fenoprofen, fenbufen, flurbiprofen, indoprofen, ketoprofen, suprofen, indomethacin, piroxicam, aspirin, salicylic acid, diflunisal, methyl salicylate, phenylbutazone, sulindac, mefenamic acid, meclofenamate sodium, tolmetin and the like;

5. Steroids such as, androgenic steroids, for example, testosterone, methyltestosterone, fluoxymesterone, estrogens for example, conjugated estrogens, esterified estrogens, estropipate, 17β-estradiol, 17β-estradiol esters such as 17β-estradiol valerate, equilin, mestranol, estrone, estriol, 17β-estradiol derivatives such as 17β-ethinyl estradiol, diesthylstilbestrol, progestational agents, such as, progesterone, 19-norprogesterone, norethindrone, norethindrone acetate, melengestrol, chlormadinone, ethisterone, medroxyprogesterone acetate, hydroxyprogesterone caproate, ethynodiol diacetate, norethynodrel, 17α-hydroxyprogesterone, dydrogesterone, dimethisterone, ethinylestrenol, norgestrel, demegestone, promegestone, megestrol acetate and the like;

6. Respiratory agents such as, theophylline and β_2-adrenergic agonists, for example, albuterol, terbutaline, metaproterenol, ritodrine, carbuterol, fenoterol,

quinterenol, rimiterol, solmefamol, soterenol, tetroquinol and the like;

7. Sympathomimetics such as, dopamine, norepinephrine, penylpropanolamine, pheylephrine, psuedoephedrine, amphetamine, propylhexedrine, arecoline and the like;

8. Antimicrobial or antiinfective agents including antibacterial agents, antifungal agents, antiparasitic agents, antimycotic agents and antiviral agents, such as, those listed in the Ashgate Handbook of Anti-Infective Agents (Ed G.W.A. Milne, Ashgate Publishing, 2000), for example, tetracyclines such as oxytetracycline; penicillins such as ampicillin; cephalosporins such as cefalotin; aminoglycosides such as kanamycin A to C, amikacin, neomycin, tobramycin, streptomycin, neamine, paromomycin, lividomycin, 2230-C, ribostamycin, xyllostasin, butirosin, 4'-deoxybutyrosin, LL-BM408a, gentamycins and nebramycin; macrolides such as erythromycin, chloramphenicol, iodides, nitrofrantoin; antifungals such as clotrimazole, miconazole, chloramphenicol, nystatin, amphotericin, fradiomycin, sulfonamides, purrolnitrin, sulfacetamide, sulfamethazine, sulfadiazine, sulfamerazine, sulfamethizole and sulfisoxazole; antivirals such as inhibitors of influenza neuraminidase and idoxuridin; clarithromycin; and other anti-infectives including nitrofurazone and the like;

9. Antihypertensive agents such as, clonidine, α-methyldopa, reserpine, syrosingopine, rescinnamine, cinnarizine, hydrazine, prazosin and the like;

10. Antihypertensive diuretics such as, chlorothiazide, hydrochlorothrazide, bendoflumethazide, trichlormethiazide, furosemide, tripamide, methylclothiazide, penfluzide, hydrothiazide, spironolactone, metolazone and the like;

11. Cardiotonics such as, digitalis, ubidecarenone, dopamine and the like;

12. Coronary vasodilators such as, organic nitrates such as, nitroglycerine, isosorbitol dinitrate, erythritol tetranitrate, and pentaerythritol tetranitrate, dipyridamole, dilazep, trapidil, trimetazidine and the like;

13. Vasoconstrictors such as, dihydroergotamine, dihydroergotoxine and the like;

14. β-blockers or antiarrhythmic agents such as, timolol pindolol, propranolol and the like;

15. Calcium antagonists and other circulatory organ agents, such as, aptopril, diltiazem, nifedipine, nicardipine, verapamil, bencyclane, ifenprodil tartarate, molsidomine, clonidine, prazosin and the like;

16. Anti-convulsants such as, nitrazepam, meprobamate, phenytoin and the like;

17. Agents for dizziness such as, isoprenaline, betahistine, scopolamine and the like;

18. Tranquilizers such as, reserprine, chlorpromazine, and antianxiety benzodiazepines such as, alprazolam, chlordiazepoxide, clorazeptate, halazepam, oxazepam, prazepam, clonazepam, flurazepam, triazolam, lorazepam, diazepam and the like;

19. Antipsychotics such as, phenothiazines including thiopropazate, chlorpromazine, triflupromazine, mesoridazine, piperracetazine, thioridazine, acetophenazine, fluphenazine, perphenazine, trifluoperazine, and other major tranquilizers such as, chlorprathixene, thiothixene, haloperidol, bromperidol, loxapine, and molindone, as well as, those agents used at lower doses in the treatment of nausea, vomiting and the like;

20. Muscle relaxants such as, tolperisone, baclofen, dantrolene sodium, cyclobenzaprine and the like;

21. Drugs for Parkinson's disease, spasticity, and acute muscle spasms such as levodopa, carbidopa, amantadine, apomorphine, bromocriptin, selegiline (deprenyl), trihexyphenidyl hydrochloride, benztropine mesylate,

procyclidine hydrochloride, baclofen, diazepam, dantrolene and the like;

22. Respiratory agents such as, codeine, ephedrine, isoproterenol, dextromethorphan, orciprenaline, ipratropium bromide, cromglycic acid and the like;

23. Non-steroidal hormones or antihormones such as, corticotropin, oxytocin, vasopressin, salivary hormone, thyroid hormone, adrenal hormone, kallikrein, insulin, oxendolone and the like;

24. Vitamins such as, vitamins A, B, C, D, E and K and derivatives thereof, calciferols, mecobalamin, and the like for dermatological use;

25. Antitumor agents such as, 5-fluorouracil and derivatives thereof, krestin, picibanil, ancitabine, cytarabine and the like;

26. Enzymes such as, lysozyme, urokinaze and the like;

27. Herb medicines or crude extracts such as, glycyrrhiza, aloe, Sikon (Lithospermi radix) and the like;

28. Miotics such as pilocarpine and the like;

29. Cholinergic agonists such as, choline, acetylcholine, methacholine, carbachol, bethanechol, pilocarpine, muscarine, arecoline and the like;

30. Antimuscarinic or muscarinic cholinergic blocking agents such as, atropine, scopolamine, homatropine, methscopolamine, homatropine methylbromide, methantheline, cyclopentolate, tropicamide, propantheline, anisotropine, dicyclomine, eycatropine and the like;

31. Mydriatics such as, atropine, cyclopentolate, homatropine, scopolamine, tropicamide, eucatropine, hydroxyamphetamine and the like;

32. Psychic energizers such as, 3-(2-aminopropyl)indole, 3-(2-aminobutyl)indole and the like;

33. Humoral agents such as, the prostaglandins, natural and synthetic, for example, PGE_1, $PGE_{2\alpha}$, and $PGF_{2\alpha}$, and the PGE_1 analog misoprostol.

34. Antispasmodics such as, atropine, methantheline, papaverine, cinnamedrine, methscopolamine and the like;

35. Antidepressant drugs such as, isocarboxazid, phenelzine, tranylcypromine, imipramine, amitriptyline, trimipramine, doxepin, desipramine, nortriptyline, proptriptyline, amoxapine, maprotiline, trazodone and the like;

36. Anti-diabetics such as, insulin, and anticancer drugs such as, tamoxifen, methotrexate and the like;

37. Anorectic drugs such as, dextroamphetamine, methamphetamine, phenylpropanolamin, fenfluramine, diethylpropion, mazindol, phentermine and the like;

38. Anti-allergenics such as, antazoline, methapyrilene, chlorpheniramine, pyrilamine, pheniramine and the like;

39. Decongestants such as, phenylephrine, ephedrine, naphazoline, tetrahydrozoline and the like;

40. Antipyretics such as, aspirin, salicylamide and the like;

41. Antimigrane agents such as, dihydroergotamine, pizotyline and the like;

42. Anti-malarials such as, the 4-aminoquinolines, alphaaminoquinolines, chloroquine, pyrimethamine and the like;

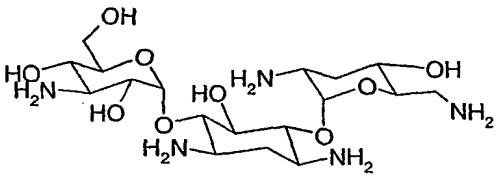
43. Anti-ulcer agents such as, misoprostol, omeprazole, enprostil, allantoin, aldioxa, alcloxa, N-methylscopolamine methylsulfate and the like;

44. Peptides such as, growth releasing factor and the like;

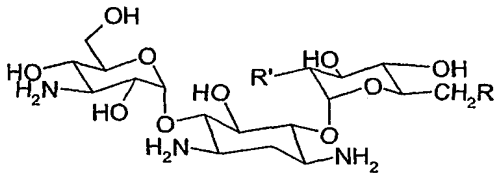
45. Anti-estrogen or anti-hormone agents such as, tamoxifen or human chorionic gonadotropin and the like.

Preferably, the pharmaceutically active moiety is an antimicrobial agent, more preferably an antibacterial agent such as an aminoglycoside, a beta-lactam antibiotic,

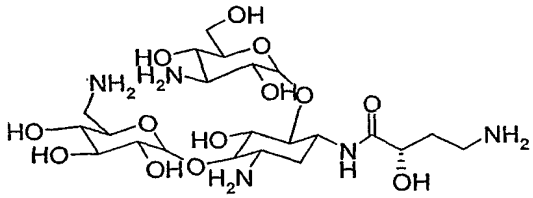
vancomycin or ciprofloxacin. Examples of aminoglycosides include the following:



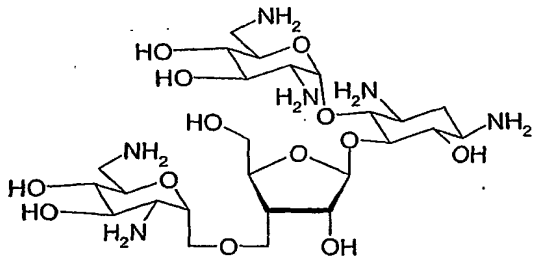
Tobramycin



	R	R'
Kanamycin A	NH_2	OH
Kanamycin B	NH_2	NH_2
Kanamycin C	OH	NH_2



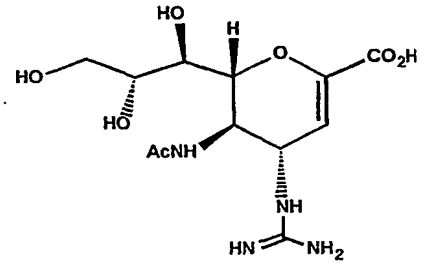
Amikacin



Neomycin

streptomycin, neamine, paromomycin, lividomycin, 2230-C, ribostamycin, xyllostasin, butirosin, 4'-deoxybutyrosin, LL-BM408a, gentamycin A, B? and nebramycin. Preferably the aminoglycoside is tobramycin, kanamycin A to C, amikacin and neomycin, more preferably tobramycin.

The antimicrobial agent may also be selected from antiviral agents, for example, nucleosides, rhinovirus capsid-binding compounds, antisense oligonucleotides, peptides, inhibitors of HIVRT and inhibitors of influenza neuraminidase, for example, a compound of Formula (A)



Compound (A)
Ac represents acetyl

; antifungal agents such as amphotericin β or azoles, for example, fluconazole or ketaconazole; or antiparasitic agents such as aspartic proteinases.

The term "linker group" is used herein in its broadest sense and refers to a functional group capable of being cleaved *in vivo* to expose the pharmaceutical moiety X. Suitable linker groups include esters, amides, ureas, thioureas, imines, acetals, ethers, phosphates, phosphate esters or diesters, thioesters, oximes and hydrazones. Preferably the linker group is an ester, amide, oxime or phosphate ester, more preferably an ester.

It will be appreciated that functional groups on the pharmaceutically active moiety form part of the linker group. Suitable points of attachment include one or more positions on the pharmaceutically active moiety containing leaving groups such as amines, hydroxyls, thiols, carboxyls, aldehydes and ketones, more preferably hydroxyls and amines. When the pharmaceutically active moiety is the aminoglycoside tobramycin, then the linker may be attached at one or more of

positions 6'', 4'', 2'', 5 and 4' which contain hydroxyl functional groups or one or more of positions 3'', 1, 3, 2' and 6' which contain amine groups. The 6''-OH and the 6'-NH_2 are the preferred points of attachment on tobramycin as they are the most accessible positions on this moiety from a synthetic standpoint.

The term "pharmacokinetic regulator" is used herein in its broadest sense and refers to a moiety which is capable of regulating residency time and the intensity of release of the pharmaceutical moiety X. The pharmacokinetic regulator may be a hydrophobic or hydrophilic moiety, preferably a hydrophobic moiety.

Suitable hydrophobic moieties include optionally substituted straight chain, branched and cyclic saturated or unsaturated hydrocarbons such as optionally substituted alkyl or optionally substituted alkenyl having 1 to 24 carbon atoms, preferably 1 to 20 carbon atoms, more preferably 1 to 16 carbon atoms, which are optionally interrupted with oxygen or nitrogen; optionally substituted aryl; or optionally substituted heterocyclyl.

The term "C_{1-24} alkyl" refers to straight chain, branched chain or cyclic hydrocarbon groups having 1 to 24 carbon atoms. Illustrative examples of straight chain or branched chain alkyl include ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, isoamyl, sec-amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, heptyl, 5-methylhexyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, octyl, 6-methylheptyl, 1-methylheptyl, 1,1,3,3-tetramethylbutyl, nonyl, 1-, 2-, 3-, 4-. 5-, 6- or 7-methyloctyl, 1-, 2-, 3-, 4- or 5-ethylheptyl, 1-, 2- or 3-

propylhexyl, decyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- and 8-methylnonyl, 1-, 2-, 3-, 4-, 5- or 6-ethyloctyl, 1-, 2-, 3- or 4-propylheptyl, undecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8- or 9-methyldecyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-ethylnonyl, 1-, 2-, 3-, 4- or 5-propyloctyl, 1-, 2- or 3-butylheptyl, 1-pentylhexyl, dodecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-methylundecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5- or 6-propylnonyl, 1-, 2-, 3- or 4-butyloctyl, 1-2-pentylheptyl and the like. Examples of cyclic alkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl and cyclodecyl and the like.

The term "C_{2-24} alkenyl" refers to straight chain, branched chain or cyclic hydrocarbon groups having from 2 to 24 carbon atoms and having in addition one or more double bonds, of either E or Z stereochemistry where applicable. Examples of straight chain and branched chain alkenyl include vinyl, allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, 1-hexenyl, 3-hexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, geranyl, farnesyl and the like. Examples of cyclic alkenyl include cyclopentenyl, 1-methyl-cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl 1,3,5-cychloheptatrienyl, 1,3,5,7-cycloocta-tetraenyl and the like.

The term "aryl" refers to single, polynuclear, conjugated and fused residues of aromatic hydrocarbons. Examples include phenyl, biphenyl, terphenyl, quaterphenyl, phenoxyphenyl, naphthyl, tetrahydronaphthyl, indane anthracenyl, dihydroanthracenyl, benzantracenyl, dibenzanthracenyl, phenanthrenyl and the like.

The term "heterocyclyl" refers to mono- or poly-cyclic saturated, partially unsaturated and unsaturated

hydrocarbon radicals containing at least one heteroatom selected from nitrogen, sulphur and oxygen. Suitable heterocyclyl groups include N-containing heterocyclic groups, such as, unsaturated 3 to 6 membered heteromonocyclic groups containing 1 to 4 nitrogen atoms, for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazolyl or tetrazolyl;

saturated 3 to 6-membered heteromonocyclic groups containing 1 to 4 nitrogen atoms, such as, pyrrolidinyl, imidazolidinyl, piperidino or piperazinyl;

unsaturated condensed heterocyclic groups containing 1 to 5 nitrogen atoms, such as, indolyl, isoindolyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl or tetrazolopyridazinyl;

unsaturated 3 to 6-membered heteromonocyclic group containing an oxygen atom, such as, pyranyl or furyl;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulphur atoms, such as, thienyl;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, such as, oxazolyl, isoxazolyl or oxadiazolyl;

saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, such as, morpholinyl;

unsaturated condensed heterocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, such as, benzoxazolyl or benzoxadiazolyl;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulphur atoms and 1 to 3 nitrogen atoms, such as, thiazolyl or thiadiazolyl;

saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulphur atoms and 1 to 3 nitrogen atoms, such as, thiazolidinyl; and

unsaturated condensed heterocyclic group containing 1 to 2 sulphur atoms and 1 to 3 nitrogen atoms, such as, benzothiazolyl or benzothiadiazolyl.

In this specification "optionally substituted" means that a group may or may not be further substituted with one or more groups selected from alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, alkynyloxy, aryloxy, carboxy, benzyloxy haloalkyl, haloalkenyloxy, haloalkynyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclic, azido, amino, alkylamino, alkenylamino, alkynylamino, arylamino, benzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, acyloxy, aldehydo, alkylsulphonyl, arylsulphonyl, alkylsulphonylamino, arylsulphonylamino, alkylsulphonyloxy, arylsulphonyloxy, heterocyclyl, heterocycloxy, heterocyclylamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio and the like. Preferably the optional substituents are alkyl, alkenyl, alkynyl, aryl, aryloxy, arylacyl, acylamino, acyloxy, nitro, hydroxy, halo and heterocyclyl.

Examples of hydrophilic moieties include oligonucleotides up to 20 nucleotides in length, peptides up to 20 amino acids in length, peptide mimics, carbohydrates, oligosaccharides and derivatives thereof.

Preferably the pharmacokinetic regulator is a hydrophobic moiety which is selected from C_{1-20} optionally substituted alkyl; C_{2-20} optionally substituted alkenyl; optionally substituted aryl; or optionally substituted heterocyclyl. The alkyl or alkenyl groups may be interrupted with O, C=O, NH, optionally substituted aryl or optionally substituted heterocyclyl and may be optionally substituted with carboxyl, optionally substituted C_{1-6} alkyl, amino or hydroxyl. The optionally substituted aryl is preferably an optionally substituted 6-membered aryl such as optionally substituted phenyl or optionally substituted biphenyl and the

heterocyclyl is preferably a 5- or 6-membered heterocyclic group such as pyridyl, indolyl, indazolyl, 2,3-dihydro-1H-indolyl, furanyl, isoxazolyl, pyrazolyl and thiofuranyl. The optional substituents on the phenyl or heterocyclyl are preferably halo, C_{1-4} alkyl, C_{1-4} alkoxy, hydroxy and OCF_3.

It will be appreciated by those skilled in the art that the prodrugs of Formula (I), (II) or (III) may be modified to provide pharmaceutically acceptable derivatives thereof at any one or more of the functional groups in the prodrugs of Formula (I), (II) or (III). Of particular interest as such derivatives are prodrugs modified at the carboxyl function, hydroxyl function or at amino groups. Thus, prodrugs of interest include alkyl esters, such as methyl, ethyl, propyl or isopropyl esters, aryl esters, such as phenyl, benzoyl esters, and acetyl esters of the prodrugs of Formula (I), (II) or (III).

The term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, hydrate, ether, ester , amide, active metabolite, analogue, residue or any other compound which is not biologically or otherwise undesirable and induces the desired pharmacological and/or physiological effect.

Pharmaceutically acceptable salts of the prodrugs of Formula (I), (II) or (III) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic acid, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining prodrugs of the invention and their pharmaceutically acceptable acid addition salts.

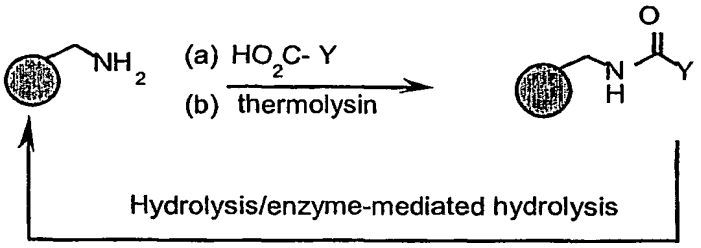
Salts derived from appropriate bases include alkali metal (*eg.* sodium), alkaline earth metal (*eg.* magnesium), ammonium, and NR_4^+ (where R is C_{1-4} alkyl) salts.

The prodrugs of the invention may be prepared by methods described herein. It will be apparent to those skilled in the art, that it may be necessary to use protecting groups to protect one or more functional groups of the pharmaceutically active moiety during the process of attaching the pharmaceutical moiety to the linker group and the pharmacokinetic regulator. See for example "Protective Groups in Organic Synthesis" by T.W. Green and P.G.M. Nuts (John Wiley & Sons, 1991).

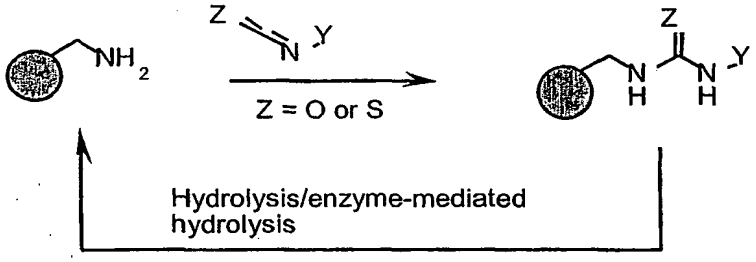
The chemistry of the linking reaction will be determined either by the nature of reactive functional groups present in the pharmaceutical moiety or the nature of reactive groups that can be introduced to the pharmaceutical moiety using a series of chemical transformations. General methods for preparing the prodrugs will now be described with reference to the nature of the functional group present in or introduced to the pharmaceutical moiety. It should be noted that many of the prodrugs described herein can be prepared using either conventional chemical methods or enzymatic methods. Enzymatic methods can in some instances provide greater selectivity than conventional methods. It will be appreciated that the invention is not limited to such methods.

<u>Pharmaceutical moieties bearing amines or to which amines can be readily introduced</u>

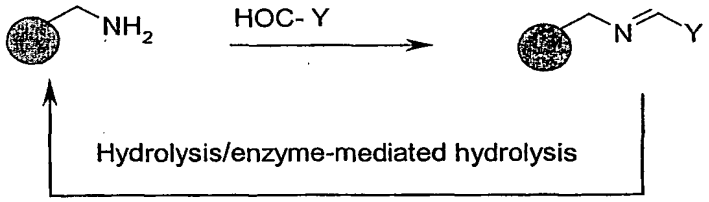
1) Amide formation
   a) Conventional chemical means
   b) Enzymatic peptide coupling using, for example, thermolysin



2) Urea or thiourea formation



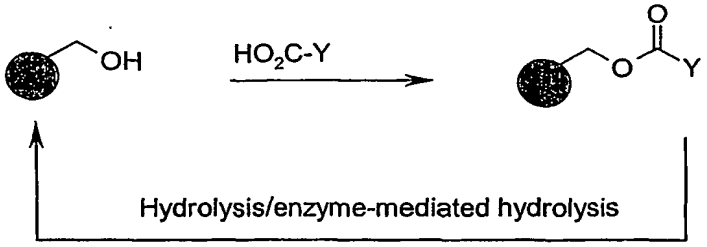
3) Imine formation



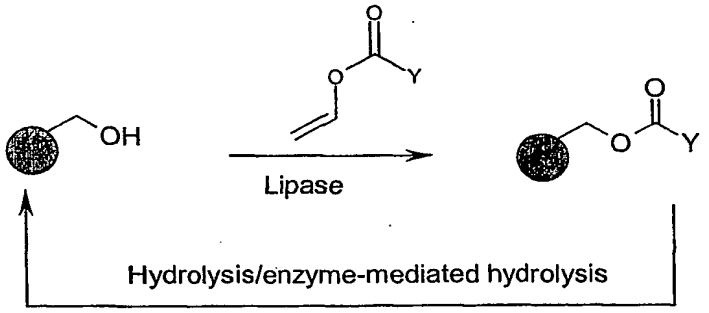
Pharmaceutical moieties bearing alcohols or to which alcohols can be readily introduced

1) Ester Formation

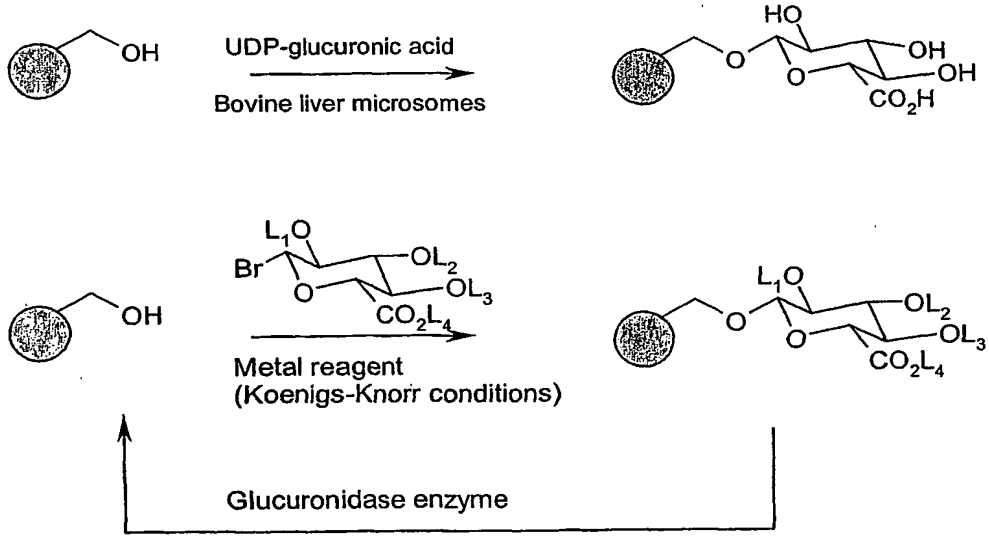
a) Conventional chemical means



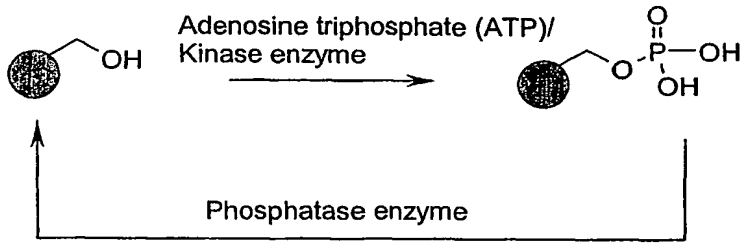
b) Transesterification using, for example, lipase enzymes



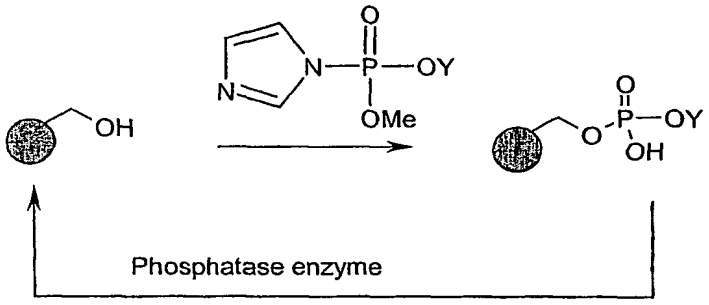
2) Acetal formation, for example, introduction of a glucuronic acid residue or a lipophile-modified version of glucuronic acid



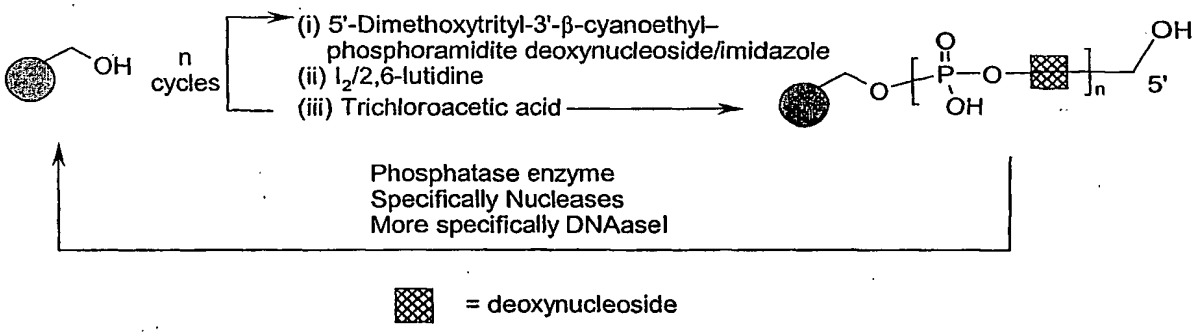
3) Phosphate formation



4) Phosphate diester formation



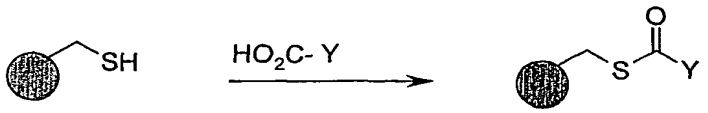
5) Oligonucleotide formation

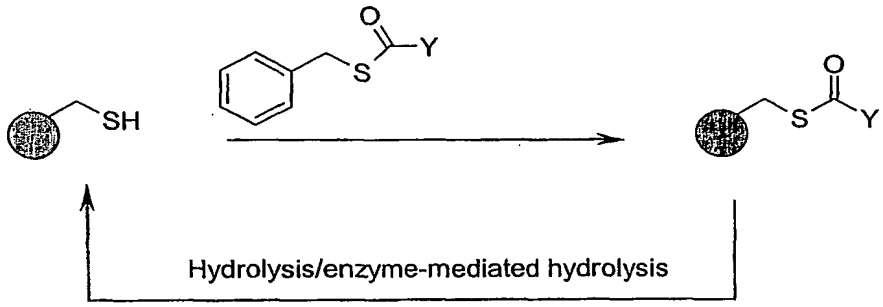


Once formed, the drug-oligonucleotide conjugate can either be base-paired with a complementary oligonucleotide according to Watson-Crick or Hoogsteen base pairing principles or can be left single stranded. The choice of whether to use double stranded, triple stranded or single stranded DNA depends on the particular phosphatase that will be used for the conversion of the prodrug into the drug.

Pharmaceutical moieties bearing thiols or to which thiols can be readily introduced

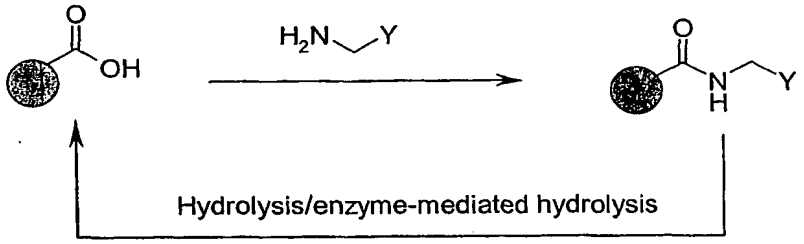
1. Thioester formation



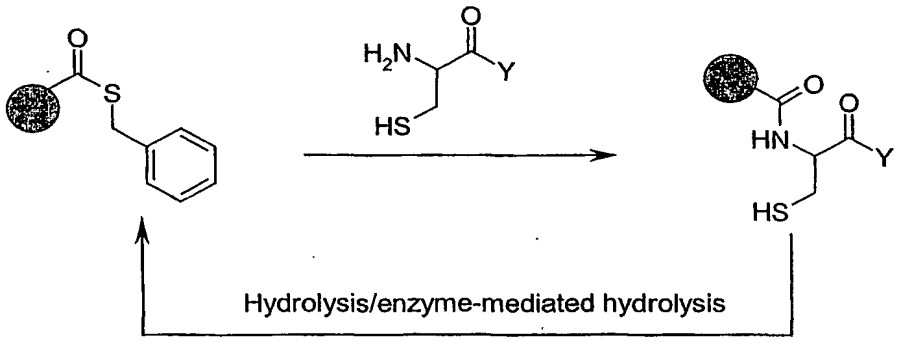


Pharmaceutical moieties bearing carboxylic acids or to which carboxylic acids can be readily introduced

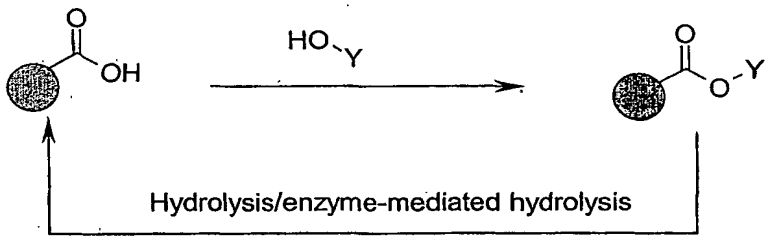
1. Amide formation



2. Amide formation (via thioesters)

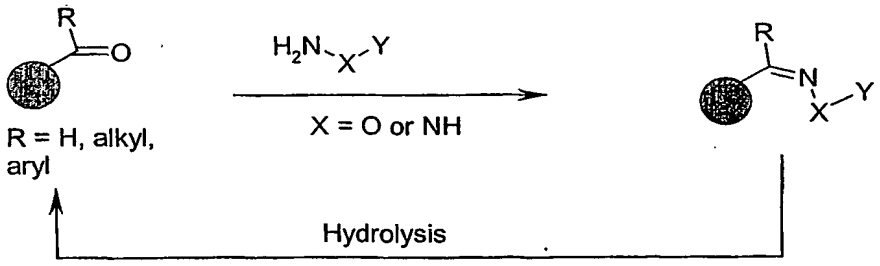


3. Ester formation



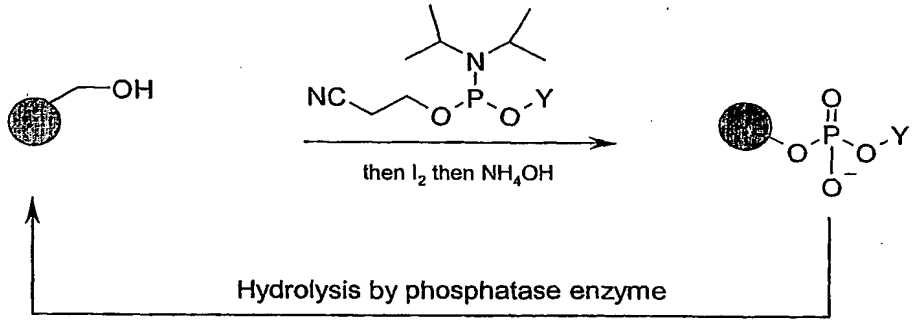
Pharmaceutical moieties bearing aldehydes and ketones or to which aldehydes and ketones can be readily introduced

1. Oxime or hydrazone formation



Pharmaceutical moieties bearing phosphate groups or their derivatives

1. Phosphate-linked dimers can be prepared as shown below.



When the pharmaceutical moiety is the preferred antimicrobial agent, then modification is preferably carried out with the primary goal of increasing residence time but this may also be accompanied by an increase in potency or therapeutic index. The choice of position at which modification should be carried out should be guided by knowledge of how the antimicrobial agent is likely to be revealed by enzymes present in the subject or knowledge of the enzymes produced by the antimicrobial agent.

Pharmaceutically acceptable salts of the prodrugs of Formula (I), (II) or (III) may be prepared according to known procedures.

For use in therapy it is preferable that the prodrugs of Formula (I), (II) or (III) are in crystalline form.

The prodrugs of Formula (I), (II) or (III) depending on the nature of the pharmaceutically active moiety may possess antimicrobial activity, preferably antibacterial activity.

The term "microbial infection" is used herein in its broadest sense and refers to any infection caused by a

microorganism and includes bacterial and viral infections. Examples of such infectious microorganisms may be found in a number of well known texts such as 'Medical Microbiology' (Greenwood, D., Slack, R., Peutherer, J., Churchill Livingstone Press, 2002); 'Mims' Pathogenesis of Infectious Disease' (Mims, C., Nash, A., Stephen, J., Academic Press, 2000); '"Fields" Virology. (Fields, B.N., Knipe, D.M., Howley, P.M., Lippincott Williams and Wilkins, 2001).

The term "microorganism" includes any microscopic organism or taxonomically related macroscopic organism within the categories algae, bacteria, fungi, protozoa, viruses and subviral agents or the like. Although, the preferable microorganism is those found in sources described above. For example, those microorganisms found in anaerobic sludge such as methanogens, eubacteria or nitrifying bacteria.

Bacterial infections include, but are not limited to, infections caused by Gram Positive Bacteria including *Bacillus cereus, Bacillus anthracis, Clostridium botulinum, Clostridium difficile, Clostridium tetani, Clostridium perfringens, Corynebacteria diphtheriae, Enterococcus (Streptococcus D), Listeria monocytogenes, Pneumoccoccal infections (Streptococcus pneumoniae), Staphylococcal* infections such as *Staphylococcus aureus* and *Streptococcal;* Gram Negative Bacteria including *Bacteroides, Bordetella pertussis, Brucella, Acinetobacter, Campylobacter, Citrobacter,* enterohaemorrhagic *Escherichia coli* (EHEC/*E. coli* 0157 : H7) enteroinvasive *Escherichia coli* (EIEC), *Enterobacter,* enterotoxigenic *Escherichia coli* (ETEC), *Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumoniae, Legionella* spp., *Moraxella catarrhalis, Neisseria gonnorrhoeae, Neisseria meningitidis, Proteus* spp., *Pseudomonas aeruginosa, Salmonella* spp., *Serratia, Vibrio cholera* and *Yersinia;* acid fast bacteria including *Mycobacterium tuberculosis, Mycobacterium avium-*

intracellulare, *Myobacterium johnei*, *Mycobacterium leprae*, atypical bacteria, *Chlamydia*, *Mycoplasma*, *Rickettsia*, *Spirochetes*, *Treponema pallidum*, *Borrelia recurrentis*, *Borrelia burgdorfii* and *Leptospira icterohemorrhagiae*; or other miscellaneous bacteria, including *Actinomyces* and *Nocardia*.

Preferably, the bacterial infection is a Gram Negative or Gram Positive infection such as infections associated with the respiratory tract (e.g. pneumonia associated with Klebsiella, mycobacterium species including tuberculosis and pseudomonas aeruginosa), urinary tract, GI tract and systemic infections caused by enteric bacteria such as *Escherichia coli*, *Proteus*, *Serratia*, *Actineobacter*, *Citrobacter*, *Enterobacter* and *Staphylococcus aureus* and plague.

Viral infections include, but are not limited to those caused by Adenovirus, Lassa fever virus (Arenavirus), Astrovirus, Hantavirus, Rift Valley Fever virus (Phlebovirus), Calicivirus, Ebola virus, Marburg Virus, Japanese encephalitis virus, Dengue virus, Yellow fever virus, Hepatitis C virus, Hepatitis G virus, Hepatitis B virus, Hepatitis D virus, Herpes simplex virus 1, Herpes simplex virus 2, Cytomegalovirus, Epstein Barr virus, Varicella Zoster Virus, Human Herpesvirus 7, Human Herpesvirus 8, Influenza virus, Parainfluenza virus, Rubella virus, Mumps virus, Morbillivirus, Measles virus, Respiratory Syncytial virus, Papillomaviruses, JC virus (Polyomavirus), BK virus (Polyomavirus), Parvovirus, Coxsackie virus (A and B), Hepatitis A virus, Polioviruses, Rhinoviruses, Reovirus, Rabies Virus (Lyssavirus), Human Immunodeficiency virus 1 and 2 and Human T-cell Leukemia virus.

Examples of viral infections include Adenovirus acute respiratory disease, Lassa fever, Astrovirus enteritis, Hantavirus pulmonary syndrome, Rift valley fever, Hepatitis E, diarrhoea, Ebola hemorrhagic fever, Marburg hemorrhagic

fever, Japanese encephalitis, Dengue fever, Yellow fever, Hepatitis C, Hepatitis G, Hepatitis B, Hepatitis D, Cold sores, Genital sores, Cytomegalovirus infection, Mononucleosis, Chicken Pox, Shingles, Human Herpesvirus infection 7, Kaposi Sarcoma, Influenza, Brochiolitis, German measles, Mumps, Measles (rubeola), Measles, Brochiolitis, Papillomas (Warts), cervical cancer, Progressive multifocal leukoencephalopathy, Kidney disease, Erythema infectiosum, Viral myocarditis, meninigitis, entertitis, Hepititis, Poliomyelitis, Cold, Diarrhoea, Rabies, AIDS and Leukemia.

Preferably, the viral infection is an orthomyxovirus or paramyxovirus infection, for example, influenza A or B, parainfluenza, mumps or Newcastle disease. More preferably the viral infection is an influenza A or B infection.

Fungal infections include, but are not limited to, infections caused by *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus versicolor, Blastomyces dermatiditis, Candida albicans, Candida dubliensis, Candida krusei, Candida parapsilosis, Candida tropicalis, Candida glabrata, Coccidioides immitis, Cryptococcus neoformans, Epidermophyton floccosum, Histoplasma capsulatum, Malassezia furfur, Microsporum canis, Mucor spp., Paracoccidioides brasiliensis, Penicillium marneffei, Pityrosporum ovale, Pneumocystis carinii, Sporothrix schenkii, Trichophyton rubrum, Trichophyton interdigitale, Trichosporon beigelii* and *Rhodotorula spp.*.

Yeast infections include, but are not limited to, infections caused by *Brettanomyces clausenii, Brettanomyces custerii, Brettanomyces anomalous, Brettanomyces naardenensis, Candida himilis, Candida intermedia, Candida saki, Candida solani, Candida tropicalis, Candida versatilis, Candida bechii, Candida famata, Candida lipolytica, Candida stellata, Candida vini, Debaromyces hansenii, Dekkera*

intermedia, Dekkera bruxellensis, Geotrichium sandidum, Hansenula fabiani, Hanseniaspora uvarum, Hansenula anomala, Hanseniaspora guillermondii Hanseniaspora vinae, Kluyveromyces lactis, Kloekera apiculata, Kluveromyces marxianus, Kluyveromyces fragilis, Metschikowia pulcherrima, Pichia guilliermodii, Pichia orientalis, Pichia fermentans, Pichia memranefaciens, Rhodotorula Saccharomyces bayanus, Saccharomyces cerevisiae, Saccharomyces dairiensis Saccharomyces exigus, Saccharomyces uinsporus, Saccharomyces uvarum, Saccharomyces oleaginosus, Saccharomyces boulardii, Saccharomycodies ludwigii, Schizosaccharomyces pombe, Torulaspora delbruekii, Torulopsis stellata, Zygoaccharomyces bailli and *Zygosaccharomyces rouxii*.

Protozoal infections include, but are not limited to, infections caused by Leishmania, Toxoplasma, Plasmodia, Theileria, Anaplasma, Giardia, Trichomonas, Trypanosoma, Coccidia, and Babesia. Specific examples include *Trypanosoma cruzi, Eimeria tenella, Plasmodium falciparum, Plasmodium vivax* or *Plasmodium ovale*.

The term "subject" as used herein refers to any animal having a disease or condition which requires treatment with a pharmaceutically-active agent. The subject may be a mammal, preferably a human, or may be a non-human primate or non-primates such as used in animal model testing. While it is particularly contemplated that the compounds of the invention are suitable for use in medical treatment of humans, it is also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, ponies, donkeys, mules, llama, alpaca, pigs, cattle and sheep, or zoo animals such as primates, felids, canids, bovids, and ungulates.

Suitable mammals include members of the Orders Primates, Rodentia, Lagomorpha, Cetacea, Carnivora, Perissodactyla and Artiodactyla. Members of the Orders

Perissodactyla and Artiodactyla are particularly preferred because of their similar biology and economic importance.

For example, Artiodactyla comprises approximately 150 living species distributed through nine families: pigs (Suidae), peccaries (Tayassuidae), hippopotamuses (Hippopotamidae), camels (Camelidae), chevrotains (Tragulidae), giraffes and okapi (Giraffidae), deer (Cervidae), pronghorn (Antilocapridae), and cattle, sheep, goats and antelope (Bovidae). Many of these animals are used as feed animals in various countries. More importantly, many of the economically important animals such as goats, sheep, cattle and pigs have very similar biology and share high degrees of genomic homology.

The Order Perissodactyla comprises horses and donkeys, which are both economically important and closely related. Indeed, it is well known that horses and donkeys interbreed.

As used herein, the term "effective amount" is meant an amount of the prodrug of Formula (I), (II) or (III) effective to preventing or treating a microbial infection in order to yield a desired therapeutic response. For example, to overcome or alleviate the effects of a microbial infection.

The term "therapeutically-effective amount" means an amount of the prodrug of Formula (I), (II) or (III) to yield a desired therapeutic response. For example, treating or preventing a microbial infection.

The specific "therapeutically-effective amount" will, obviously, vary with such factors as the particular microbial infection being treated, the physical condition of the subject, the type of animal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulation employed and the structure of the compound or its derivatives.

Generally, the terms "treating", "treatment" and the like are used herein to mean affecting a subject, tissue or cell to obtain a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a microbial infection or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure of a microbial infection. "Treating" as used herein covers any treatment of, or prevention of a microbial infection in a vertebrate, a mammal, particularly a human, and includes: (a) preventing the microbial infection from occurring in a subject that may be predisposed to the microbial infection, but has not yet been diagnosed with the microbial infection; (b) inhibiting the microbial infection, i.e., arresting its development; or (c) relieving or ameliorating the effects, i.e., cause regression of the symptoms of the microbial infection.

The prodrugs of the invention may also be used in diagnostic methods, in particular methods for the detection of microbial infections such as the influenza virus. For use in such methods it may be advantageous to link a prodrug of the invention to a label, such as a radioactive, fluorescent or chemiluminescent label.

Methods of diagnosis for which the prodrugs of the invention are suitable are described, for example, in our earlier applications PCT/AU97/00109 and PCT/AU97/00771.

It will be further appreciated that the amount of the prodrug of the invention required for use in treatment will vary not only with the particular prodrug selected but also with the route of administration, the nature of the condition being treated, and the age and condition of the subject, and will ultimately be at the discretion of the attendant physician or veterinarian. In general however, a suitable dose will be in the range of from about 0.001 to 100 mg/kg of bodyweight per day, preferably in the range of

0.001 to 1 mg/kg/day, most preferably in the range of 0.002 to 0.1 mg/kg/day.

Treatment is preferably commenced before or at the time of infection and continued until microorganism is no longer present. However the prodrugs are also effective when given post-infection, for example, after the appearance of established symptoms.

Suitably treatment is given on one or two occasions, preferably only once only for treatment and preferably once per week for prophylaxis.

The prodrug is conveniently administered in unit dosage form, for example containing 1 to 100 mg, more conveniently 0.1 to 10 mg, most conveniently 0.1 to 5 mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, the prodrug of the invention may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not being deleterious to the subject thereof.

The prodrugs of the invention may also be used in combination with other therapeutic and/or prophylactic agents, for example other antimicrobial or antiinfective agents. In particular the prodrugs of the invention may be employed with other antibacterial agents.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier form part of the invention.

Suitable therapeutic and/or prophylactic agents for use in such combinations include other antimicrobial agents, in particular antibacterial agents such as combinations of trimethoprim and sulfonamide; bacitracin and polymyxin B-

neomycin; imipenem and fluoroquinolone; and beta-lactam and aminoglycosides.

The individual components of such combinations may be administered either separately, sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the prodrugs of the invention are used with a second therapeutic and/or prophylactic agent active against the same microorganism, the dose of each prodrug may either be the same as or different from that employed when each prodrug is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or those in a form suitable for administration to the respiratory tract (including the nasal passages) for example by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units, and may be prepared by any of the methods well known in the art of pharmacy. These methods include the step of bringing into association the prodrug with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the prodrug; as a powder or granules; as a solution, a suspension or as an emulsion. The prodrug may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may for example be in the form of

aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles, which may include edible oils, or preservatives.

The prodrugs according to the invention may also be formulated for parenteral administration by injection, for example bolus injection, or continuous infusion, and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilising and/or dispersing agents. Alternatively, the prodrug may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, *eg.* sterile, pyrogen-free water, before use.

For topical administration to the epidermis the prodrugs according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base, and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising the prodrug in a flavoured base, usually sucrose and gum acacia or gum tragacanth; pastilles comprising the prodrug in an inert base such as gelatin or sucrose and gum acacia; and mouthwashes comprising the prodrug in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the prodrug with the softened or melted carrier(s) followed by chilling and shaping moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For administration to the respiratory tract, including intranasal administration, the neuraminidase inhibitors may be administered by any of the methods and formulations employed in the art for administration to the respiratory tract.

Thus in general the prodrugs may be administered in the form of a solution or a suspension or as a dry powder.

Solutions and suspensions will generally be aqueous, for example prepared from water alone (for example sterile or pyrogen-free water) or water and a physiologically acceptable co-solvent (for example ethanol, propylene glycol or polyethylene glycols such as PEG 400).

Such solutions or suspensions may additionally contain other excipients for example preservatives (such as benzalkonium chloride), solubilising agents/surfactants such as polysorbates (*eg.* Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided

in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a dropper or pipette this may be achieved by the subject administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant, such as a chlorofluorocarbon (CFC), for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluoroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the prodrugs may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form, for example in capsules or cartridges of *eg.* gelatin, or blister packs from which the powder may be administered by means of an inhaler.

In formulations intended for administration to the respiratory tract, including intranasal formulations, the prodrug will generally have a small particle size, for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired, formulations adapted to give sustained release of the prodrug may be employed.

Preferably the prodrugs of the invention are administered to the respiratory tract by inhalation,

insufflation or intranasal administration, or a combination thereof.

"Relenza" is administered by oral inhalation as a free-flow powder via a "Diskhaler" (trade mark of Glaxo Wellcome plc). A similar formulation would be suitable for the present invention.

It will be appreciated that the inhaler may also be in the form of a meter dose aerosol inhaler.

BRIEF DESCRIPTION OF THE DRAWINGS

In the Examples, reference will be made to the accompanying drawings in which:

Fig. 1 is a graph of tobramycin concentration relative to kanamycin A 168 hours post dose;

Fig. 2 is the MS/MS spectra for compound 26;

Fig. 3 is the MS/MS spectra for compound 29; and

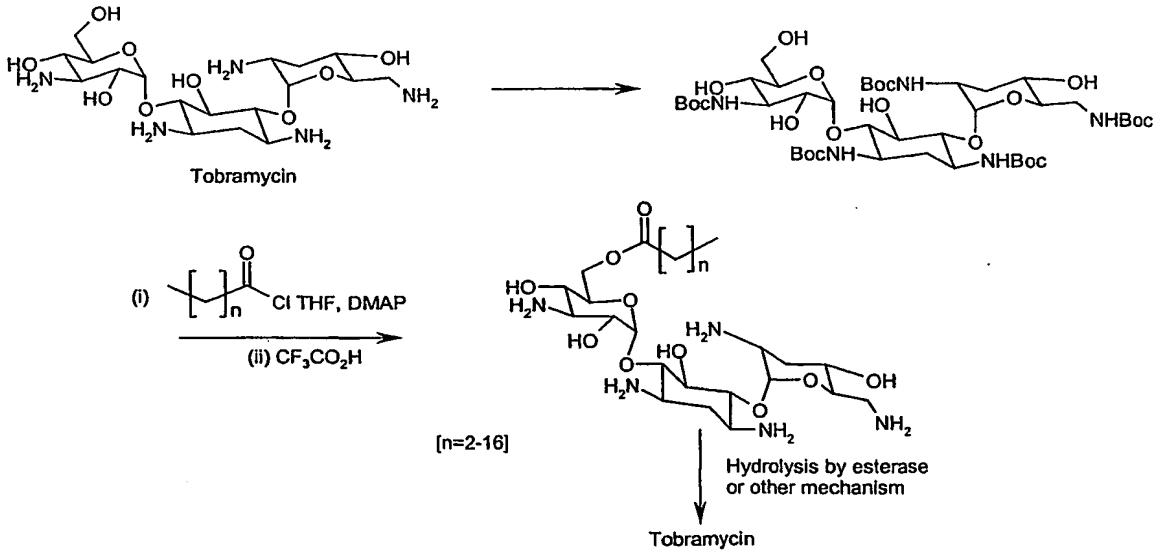
Fig. 4 is the MS/MS spectra for compound 30.

EXAMPLES

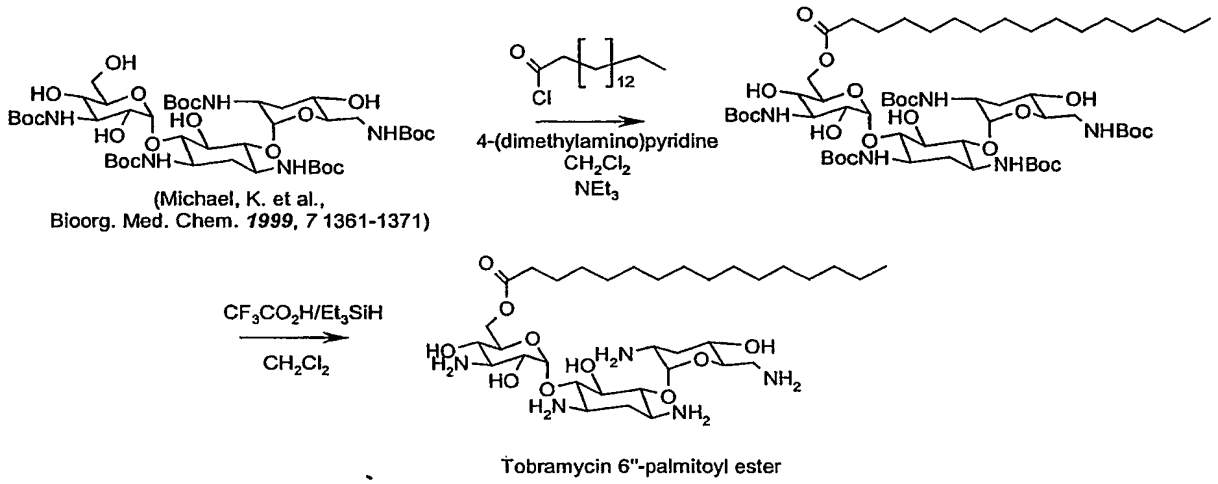
The invention will now be described in detail by way of reference only to the following non-limiting examples.

The examples describe methods for the preparation of hydrolysis-activated prodrugs of aminoglycoside antibiotic tobramycin.

Example 1: Ester linkage - activation by hydrolysis



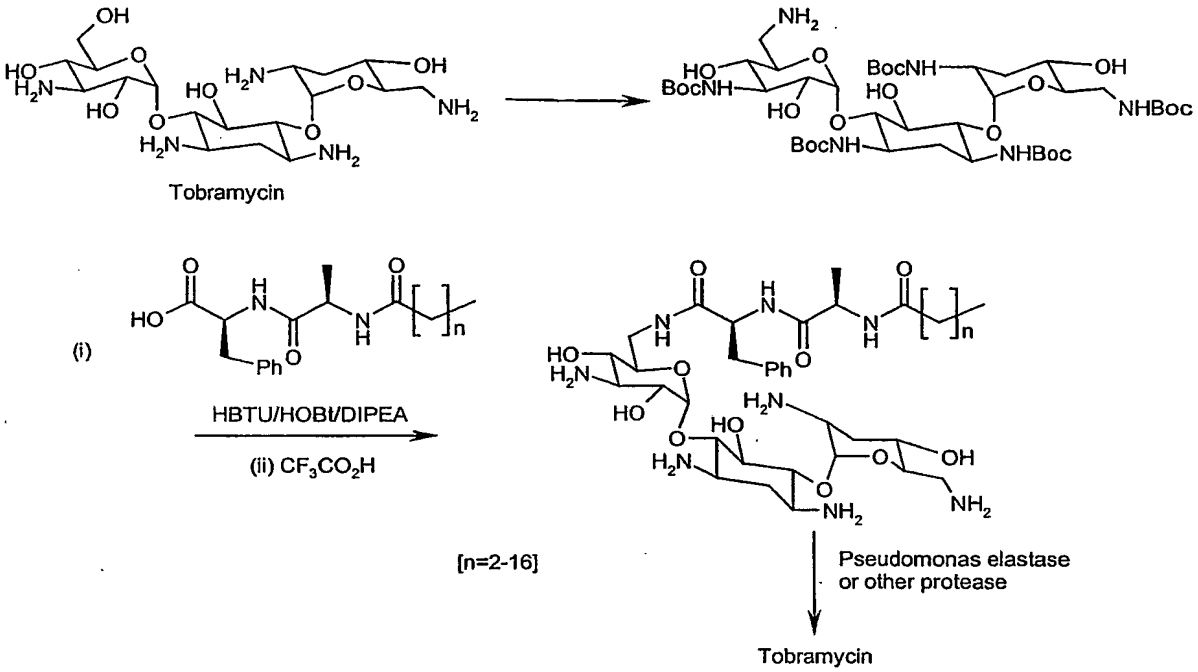
Example 2: Tobramycin 6''-palmitoyl ester



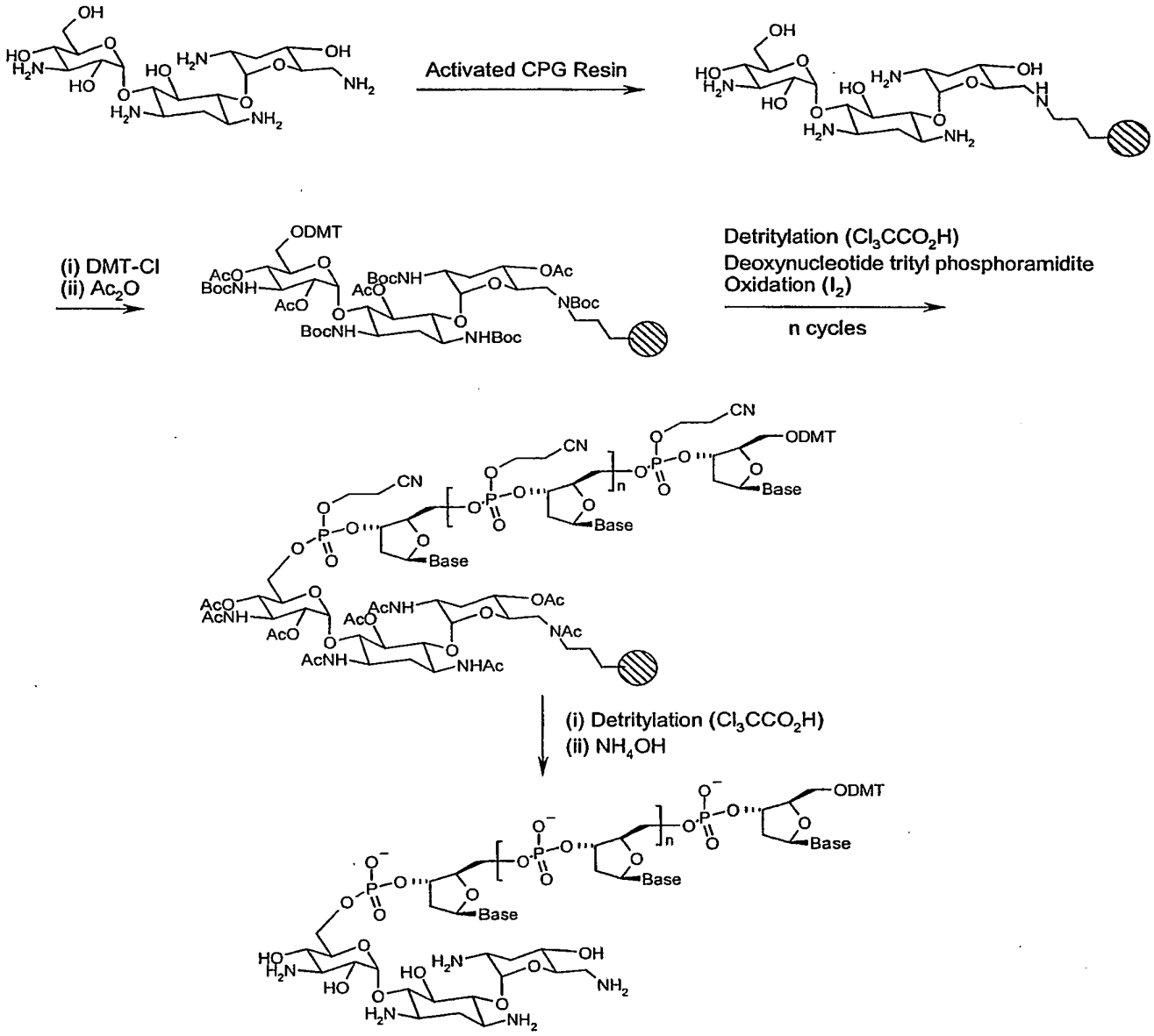
Boc-tobramycin (68mg, 0.07mmole) in dichloromethane (15mL) was treated with 4-(dimethylamino)pyridine (13.2mg, 0.108mmole) and palmitoyl chloride (27.5mg, 0.1mmole). The reaction mixture was stirred at room temperature for 30 minutes then treated with triethylamine (143µL, 1.08mmole) and stirred for a further 18 hours at room temperature. The dichloromethane was then removed under reduced pressure and the residue dissolved in 1:1 water:diethyl ether. The ether layer was washed with water and saturated aqueous sodium bicarbonate then dried, filtered and concentrated to give 61mg of residue.

The residue was dissolved in dichloromethane (3mL) and then treated with triethylsilane (100µL) and trifluoroacetic acid (3mL). After 2 hours stirring at room temperature, the volatiles were removed under reduced pressure and the residue purified by preparative LCMS. Calculated for $C_{34}H_{67}N_5O_{10}$, 705. Found (ESMS) 706 ([M+H]+).

Example 3: Amide linkage - activation by hydrolysis



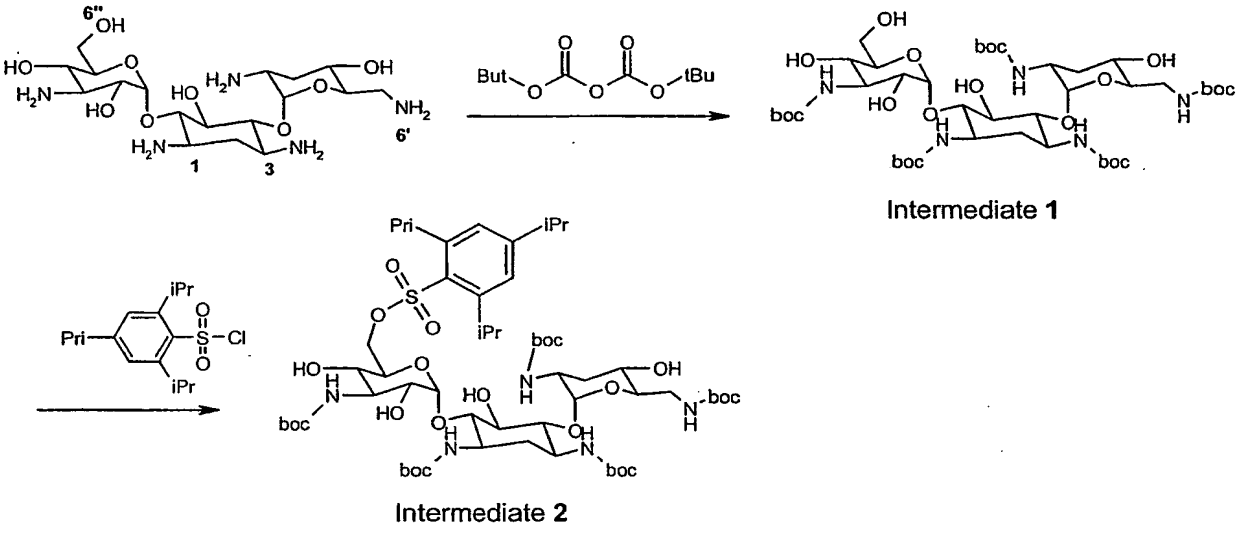
Example 4: Oligodeoxynucleotide formation



Example 5: Preparation of aminoglycoside esters, amides and oximes and aminoglycoside dimers

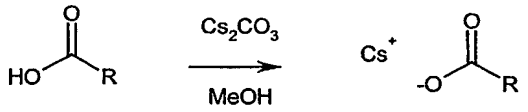
Aminoglycoside intermediates **1** and **2** were prepared as shown in **Scheme 1** by adaptation of procedures previously published by Michael, K. *et al.*, *Bioorg. Med. Chem.* **1999**, 7 1361-1371.

Scheme 1.



General method for preparation of cesium salts from carboxylic acids.

Intermediates **3** were prepared by a modified method previously described by *J. Org. Chem*, **1987**,52 ,4230-4234



Intermediates 3

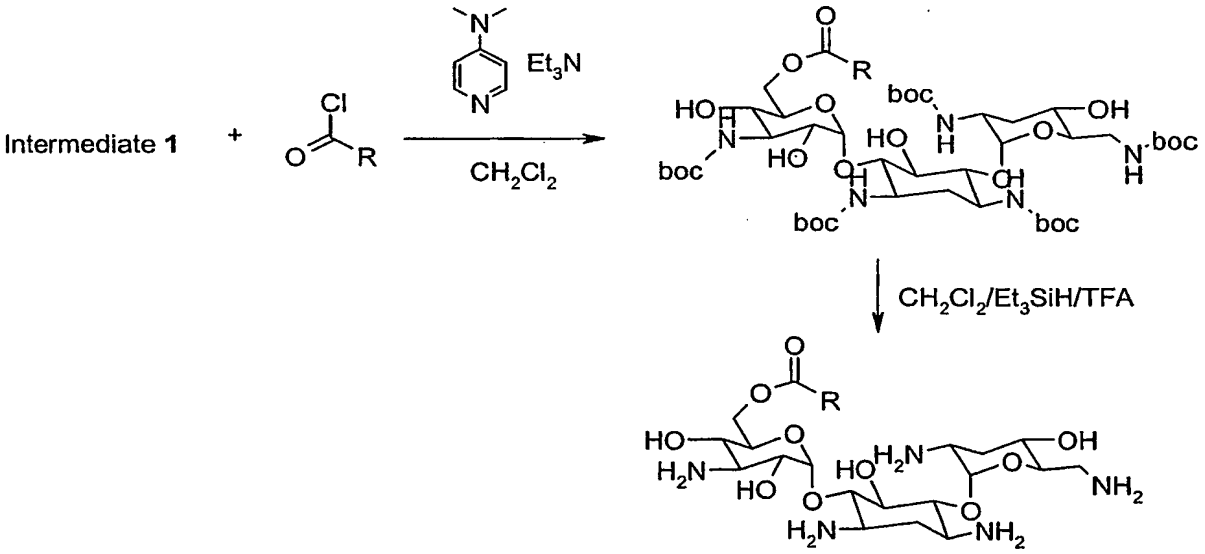
To a solution of cesium carbonate (3mmol) in anhydrous MeOH (20mL) was added a solution of carboxylic acid (9mmol) in MeOH (9mL). The mixture was stirred under argon for 30mins

and solvent evaporated. The resulting white solid was washed thoroughly with ether to remove any un-reacted acid and the solid material was dried under vacuum (1mmHg) at 80°C over P_2O_5 then stored under dry nitrogen.

Method A:

To a solution consisting of the appropriate intermediate **3** (248µmol) in anhydrous DMF (5mL) was added a solution of intermediate **2** (40.5µmol) in anhydrous DMF (2mL). The mixture was stirred at 75° C for 24h under a dry nitrogen atmosphere. The reaction was monitored by LCMS. Upon completion of the reaction, the solvent was removed *in vacuo* to leave a sticky residue, which was re-dissolved in CF_3CO_2H (2mL) at room temperature. After 5mins the CF_3CO_2H was removed under a stream of nitrogen, the residues were re-dissolved in H_2O (10mL) and the solution lyophilized. The product was isolated by reverse phase chromatography, conditions for which are provided in Table 5.

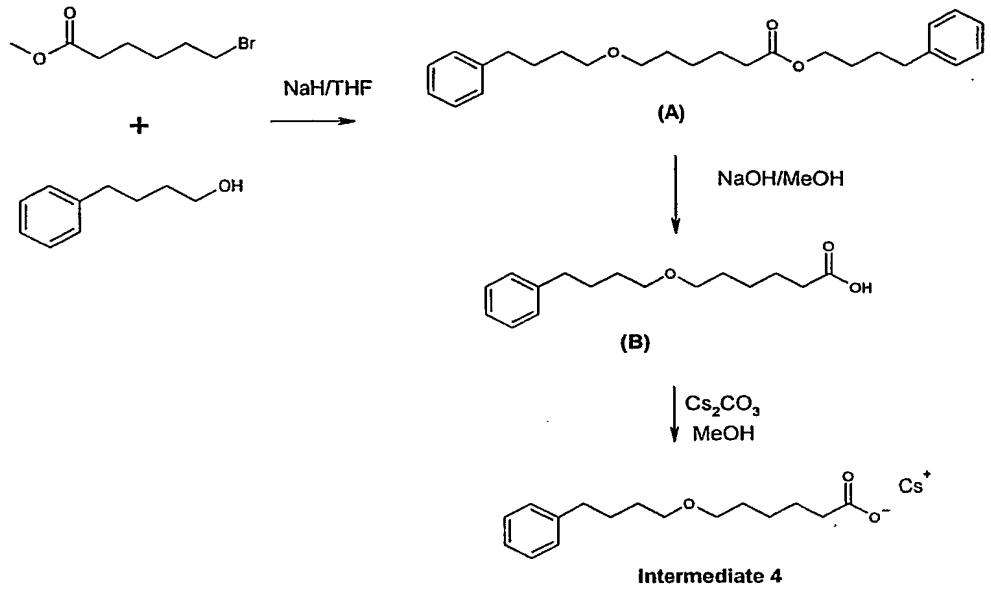
Method B:



Intermediate **1** (0.07mmole) was dissolved in dry dichloromethane (15ml) and N,N-dimethylaminopyridine (DMAP) (0.108mmole) was added followed by the appropriate acid chloride (0.1mmole). After stirring at ambient temperature for 30min., triethylamine (0.108mmole) was added and the reaction was stirred for 16h at room temperature. The solvent was removed under vacuum and the residue was partitioned between a mixture of ether (10ml) and water (10ml). The ether phase was separated, washed with water, then saturated sodium bicarbonate solution, dried over anhydrous sodium sulphate and concentrated *in vacuo*.

The crude residue was dissolved in dichloromethane (3ml) and triethylsilane (TES) (100μl) was added followed by trifluoroacetic acid (3ml). The solution was stirred for 2 h at room temperature then evaporated to dryness under vacuum at 40°C. The crude product was purified by reverse phase chromatography.

Intermediate 4:



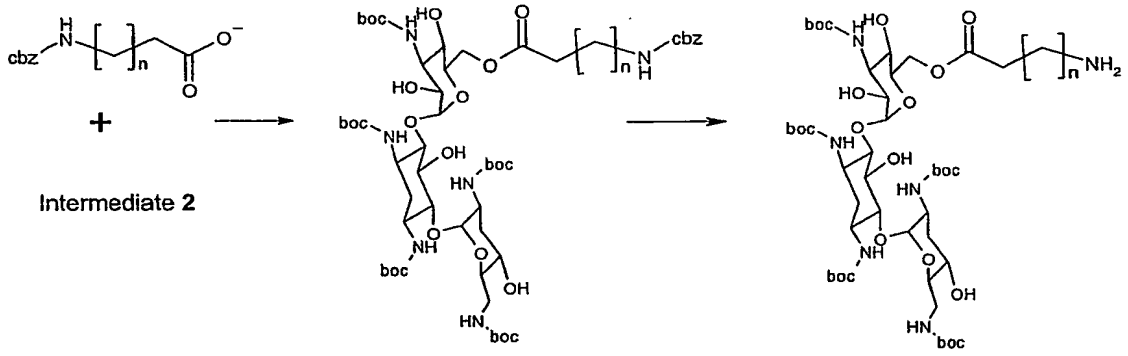
Intermediate 4

NaH (60% dispersion in oil; 6.37 mmol) was added portions wise to a solution of 4-phenyl-1-butanol (5.1mmol) and 6-bromohexanoate (5.1mmol) in anhydrous THF (10mL) under an inert atmosphere. The suspension was stirred and heated on reflux for 27h at 70°C. To the reaction mixture was cooled then treated with H_2O (20mL) then extracted with diethyl ether (20mL x 3). The combined organic extracts were dried over $MgSO_4$, filtered then concentrated to dryness to obtain 6-(4-phenyl-butoxy)-hexanoic acid 4-phenyl-butyl ester (**A**) as an orange oil. Calculated for compound (**A**) $C_{26}H_{36}O_3$ 396; found m/z= 396.91 $[M+H]^{1+}$. This material was dissolved in MeOH (1mL) and 1N NaOH (1mL) and stirred at room temperature. After 4h, the reaction mixture was concentrated to dryness to obtain a colourless residue. 6-(4-Phenyl-butoxy)-hexanoic acid (**B**) was isolated by partitioning the residues between H_2O (50 mL) and $CHCl_3$ (50 mL). The organic layer was removed and the aqueous layer washed with two more aliquots of $CHCl_3$ (50mL). The aqueous layer was lyophilized to produce a white

solid. Calculated for compound (**B**) $C_{16}H_{24}O_3$ 264; found m/z 264.37 $[M+H]^{1+}$. Formation of the cesium salt (Intermediate **4**) was performed using the generalised procedure described for intermediates **3**.

Compound **14** was prepared by adopting **Method A.**

It would be reasonable to expect that the above methodology used to introduce esters to the 6''-position of tobramycin could also be extrapolated to other aminoglycosides equipped with appropriate leaving groups as suitable positions. For example, Michael and co-workers referenced to above have shown that leaving groups can be introduced to each of neomycin, kanamycin and tobramycin and these displaced with suitable nucleophiles. Method A described above could also be applied to these aminoglycosides.



Compounds of Formula (**IV**) were prepared as described in **Method A.** The products were dissolved in minimum amount of MeOH and was purified by flash chromatography (SiO_2, 2.5% MeOH in CH_2Cl_2) prior to removal of the Cbz group. The purified material was treated with MeOH and 10% Pd/C then stirred at room temperature under hydrogen gas at atmospheric pressure for 16h. The solvent was then removed under reduced pressure to afford intermediate **5** or **6** as white powders.

Method C:

A solution containing intermediate **5** or **6** (0.093 mmol), diacid (0.038 mmol) and benzotriazol-1-yloxytri(dimethylamino)phosphonium (BOP) (0.093 mmol) in dried N,N-dimethylacetamide (DMA) (2.5 mL) was stirred for 5mins prior to addition of diisopropylethylamine (DIEA) (0.76 mmol). The reaction mixture was heated at 75°C for 24h under nitrogen. The reaction was monitored by LCMS and upon completion, the solvent was removed *in vacuo* to afford a dark residue. The resulting material was treated with CF_3CO_2H (2mL) at room temperature and after 5mins the solvent was evaporated generally to afford an oil. The product was isolated by preparative reverse phase chromatography, conditions for which are provided in Table 5.

Method D:

To a solution of intermediate **5** or **6** (0.093 mmol) and DMAP (0.093 mmol) in anhydrous DMA (2mL) was added the appropriate diisocyanate (0.038 mmol). The mixture was allowed to stir for 48h under nitrogen at 75°C. The solvent was removed *in vacuo* and the residue was treated with CF_3CO_2H (5mL) for 5mins. The CF_3CO_2H was removed to obtain a residue which was redissolved in H_2O (10mL) and freeze-dried to obtain crude product. The product was purified by preparative reverse phase chromatography, conditions for which are described in Table 5.

Method E:

To a chilled solution of BOC-L-Asp(OtBu)-OH. DCHA (0.178mmol) and BOP (0.214mmol) in DMF (1mL) was added a solution of tobramycin (0.214mmol) in H_2O (2mL). The mixture was stirred at room temperature under nitrogen for 16h and solvent removed under vacuum to afford a colourless oil. The

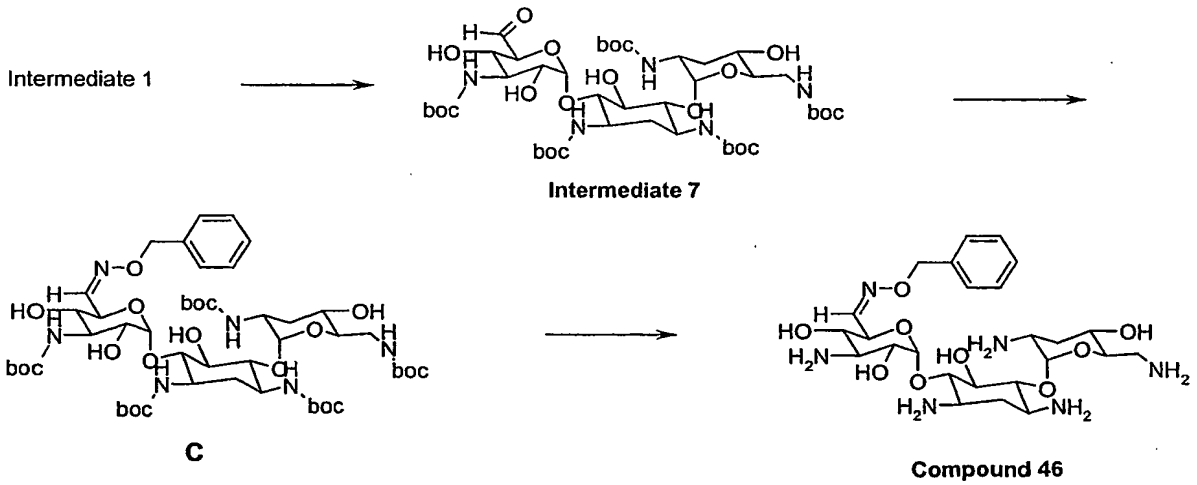
crude material was isolated by preparative reverse phase HPLC and the purified material was treated with 50% TFA/CH_2Cl_2 (2mL) and 5% Et_3SiH (100μL) for 4h. The solvent was removed *in vacuo* and the residue was dissolved in H_2O (10mL) then lyophilized overnight to afford a sandy solid.

Method F:

The di-cesium salt was prepared by the method described for intermediate **3**. This intermediate (0.05mmole) was added to a solution of intermediate 2 (0.1mmole) in DMA (3ml). The reaction was stirred and heated at 80°C for 72h under argon. The residue was triturated with water and filtered. After washing with more water, the residue was dissolved in CH_2Cl_2, dried over Na_2SO_4 and the solution evaporated to dryness.

The residue was dissolved in CF_3CO_2H (8mL), stirred for 5 minutes and evaporated to dryness under vacuum with the minimum of heat. The product was then purified by preparative reverse phase chromatography.

General method for preparation of tobramycin oximes at the 6''-position.

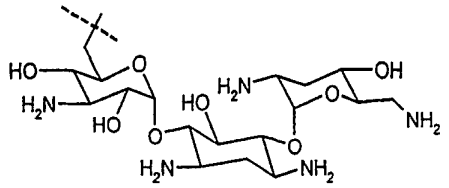


Intermediate 1 (0.052mmole) was stirred in dry CH_2Cl_2 (2ml) and tetrahydrofuran (2ml) at 0°C before the addition of Dess-Martin Periodinane (0.077mmole). The reaction was allowed to warm to room temperature over 2 hours and concentrated *in vacuo*. The crude product was purified by chromatography on silica eluting with 10%MeOH in CH_2Cl_2. Rf=0.47 (10%MeOH/ CH_2Cl_2). Calculated for Intermediate 7 $C_{43}H_{75}N_5O_{19}$ 965; found m/z 866.15 $[(M\text{-boc})+H]^{1+}$.

Intermediate 7 (0.050mmole) and O-benzylhydroxylamine hydrochloride salt (0.2mmole) were stirred in a biphasic system with CH_2Cl_2 (3ml) and 10mM ammonium acetate (3ml). After 2 days the CH_2Cl_2 layer was separated and the aqueous phase extracted with CH_2Cl_2 (3x10ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*.

The crude residue was dissolved in CH_2Cl_2 (2ml) followed by trifluoroacetic acid (2ml). The solution was stirred for 1 h at room temperature, evaporated to dryness *in vacuo* and triturated with hexane/ CH_2Cl_2 (1:1 2x10ml). The crude product was purified by reverse phase chromatography.

Table 2: Synthetic & Structural Details for Aminoglycoside Monoesters

AG = 

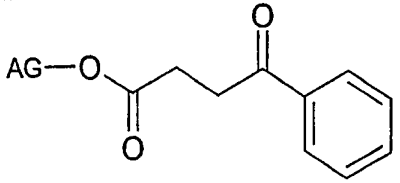
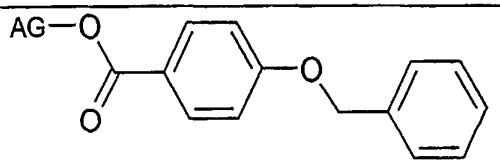
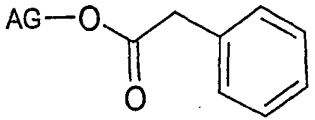
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
1	Cesium 1,16 - hexadecanedioate	2	A	
2	Cesium 3-benzoylpropionate	2	A	
3	Cesium 4-benzyloxybenzoate	2	A	
4	Cesium phenylacetate	2	A	

Table 2 cont: Synthetic & Structural Details for Aminoglycoside Monoesters

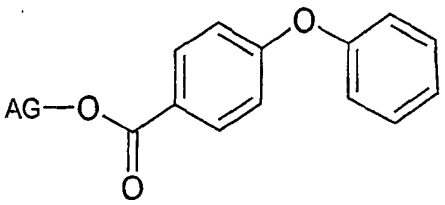
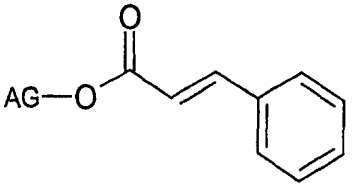
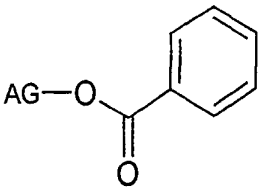
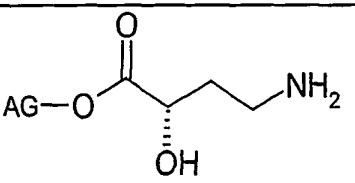
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
5	Cesium 4-phenoxylbenzoate	**2**	A	
6	Cesium *trans*-cinnamate	**2**	A	
7	Cesium benzoate	**2**	A	
8	Cesium BOC-amino-3-hydroxylbutanoate	**2**	A	

Table 2 cont: Synthetic & Structural Details for Aminoglycoside Monoesters

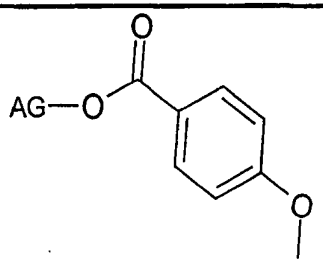
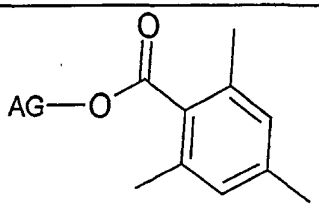
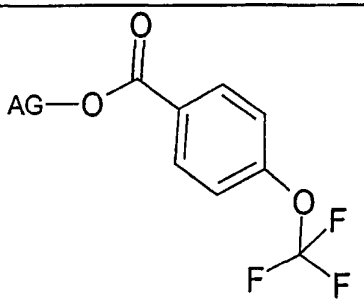
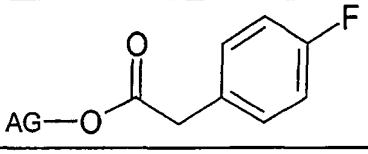
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
9	Cesium 4-methoxybenzoate	**2**	A	
10	Cesium 2,4,6-trimethylbenzoate	**2**	A	
11	Cesium 4-(trifluoromethoxy) benzoate	**2**	A	
12	Cesium 4-fluorophenylacetate	**2**	A	

Table 2 cont: Synthetic & Structural Details for Aminoglycoside Monoesters

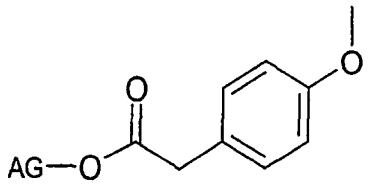
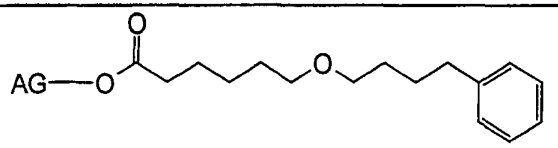
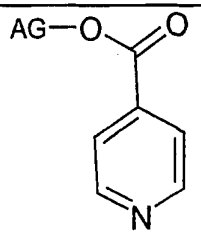
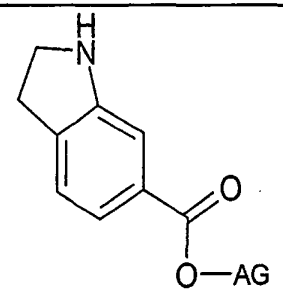
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
13	Cesium 4-methoxylphenyl acetate	**2**	A	
14	Cesium 6-(4-phenyl-butoxy)-hexanoate (Intermediate 4)	**2**	A	
15	Cesium isonicotinate	**2**	A	
16	Cesium indole-6-carboxylate	**2**	A	

Table 2 cont: Synthetic & Structural Details for Aminoglycoside Monoesters

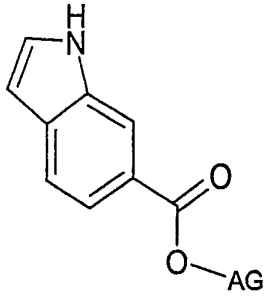
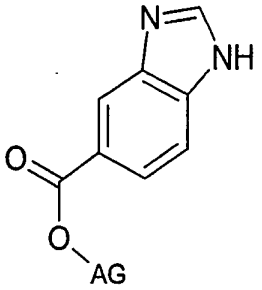
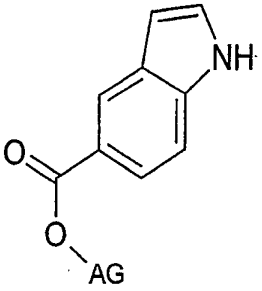
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
17	Cesium indole-6-carboxylate	**2**	A	
18	Cesium 5-benzimidazole carboxylate	**2**	A	
19	Cesium indole-5-carboxylate	**2**	A	

Table 2 cont: Synthetic & Structural Details for Aminoglycoside Monoesters

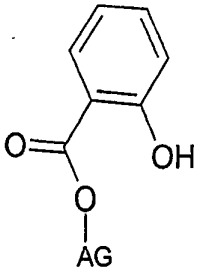
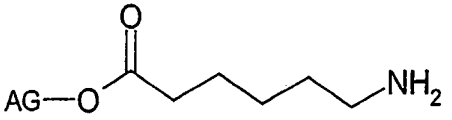
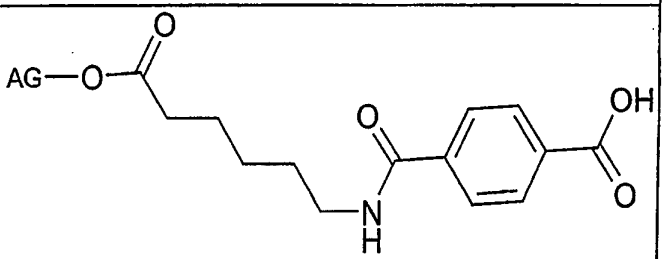
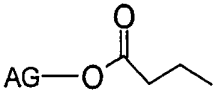
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
20	Cesium salicylate	**2**	A	
21 (Intermediate 6)	Cesium Cbz-aminocaproate	**2**	A	
22	Cesium terephthalate	**6**	C	
23	Cesium butyrate	**2**	A	
24	Cesium heptadecanoate	**2**	A	

Table 2 cont: Synthetic & Structural Details for Aminoglycoside Monoesters

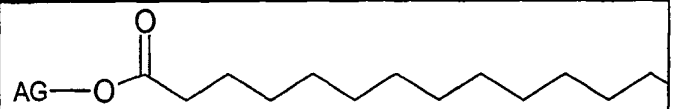
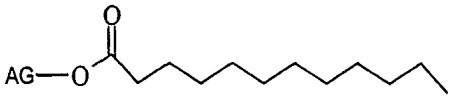
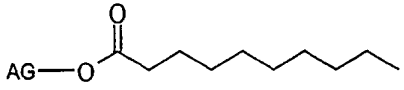
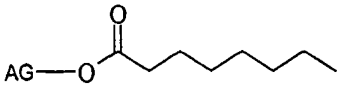
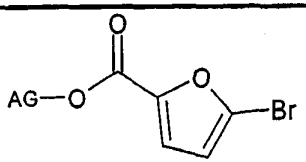
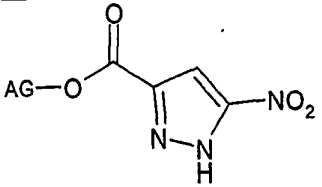
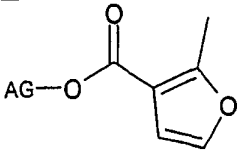
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
25	Palmitoyl chloride	1	B	
26	Cesium dodecanoate	2	A	
27	Cesium decanoate	2	A	
28	Cesium octanoate	2	A	
40	Cesium 5-Bromo-furan-2-carboxylate	2	A	
41	Cesium 5-Nitro-1H-pyrazole-3-carboxyate	2	A	
42	Cesium 2-Methyl-furan-3-carboxylate	2	A	

Table 2 cont: Synthetic & Structural Details for Aminoglycoside Monoesters

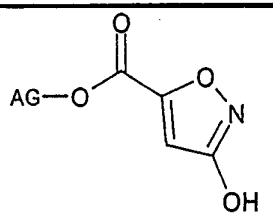
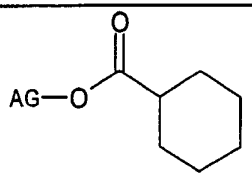
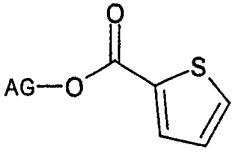
Compound	Modification reagent (Intermediates 3)	Amino-glycoside Intermediate	General Method	
43	Cesium 3-Hydroxy-isoxazole-5-carboxylate	**2**	A	
44	Cesium Cyclohexane carboxylate	**2**	A	
45	Cesium Thiophene-2-carboxylate	**2**	A	

Table 3: Synthetic & Structural Details for Aminoglycoside Esters, Amides and Oximes

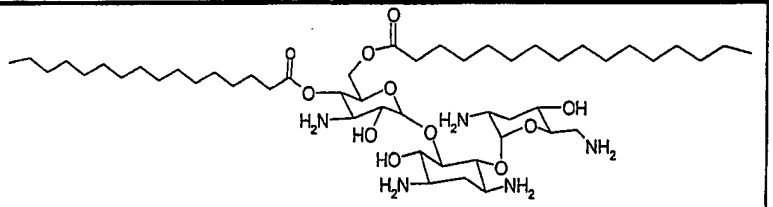
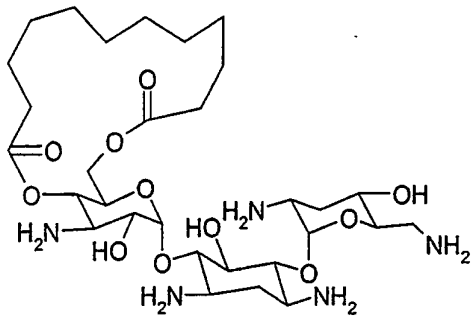
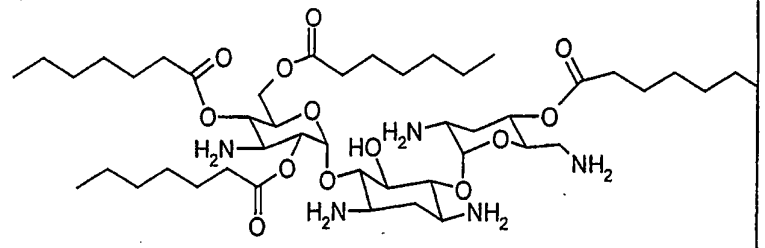
Compound	Modification reagent	Amino-glycoside Intermediate	General Method	Compound Structure
29	Palmitoyl chloride	**1**	B	
30	Dodecanoyl chloride	**1**	F	
31	Heptanoyl chloride	**1**	B	

Table 3 cont: Synthetic & Structural Details for Aminoglycoside Esters, Amides and Oximes

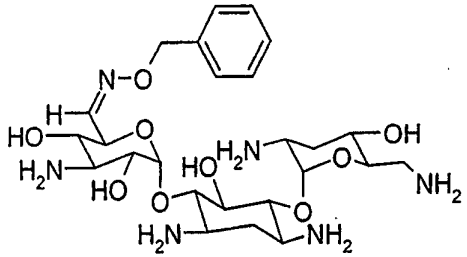
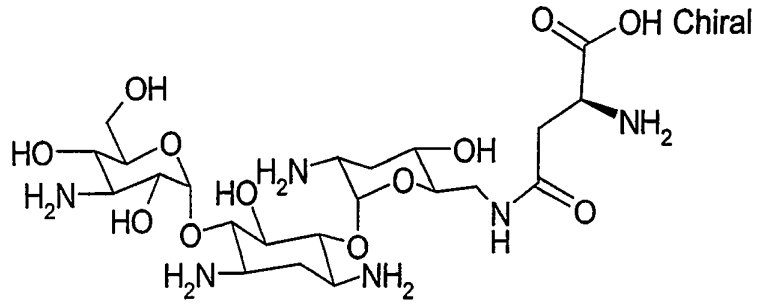
Compound	Modification reagent	Amino-glycoside Intermediate	General Method	Compound Structure
46	O-Benzylhydroxylamine	7	N/A	
32	Obu^t[BOC-L-Asp-OtBu]	Tobramycin	E	

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Table 3 cont: Synthetic & Structural Details for Aminoglycoside Esters, Amides and Oximes

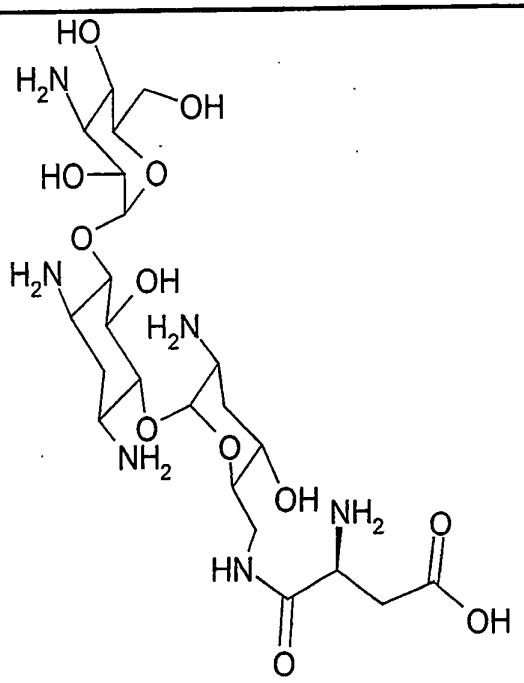
Compound	Modification reagent	Amino-glycoside Intermediate	General Method	Compound Structure
33	BOC-L-Asp(OtBu)-OH..DCHA	Tobramycin	E	

Table 4: Synthetic & Structural Details for Dimeric Aminoglycosides

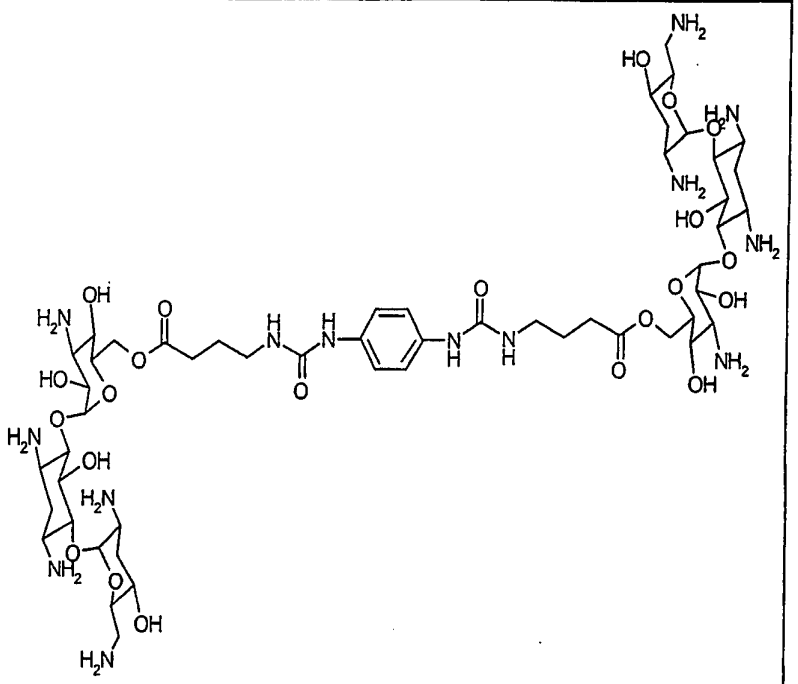
Compound	Modification reagent	Amino-glycoside Intermediate	General Method	Compound Structure
34	1,4-Phenyldiisocyanate	5	D	

Table 4 cont: Synthetic & Structural Details for Dimeric Aminoglycosides

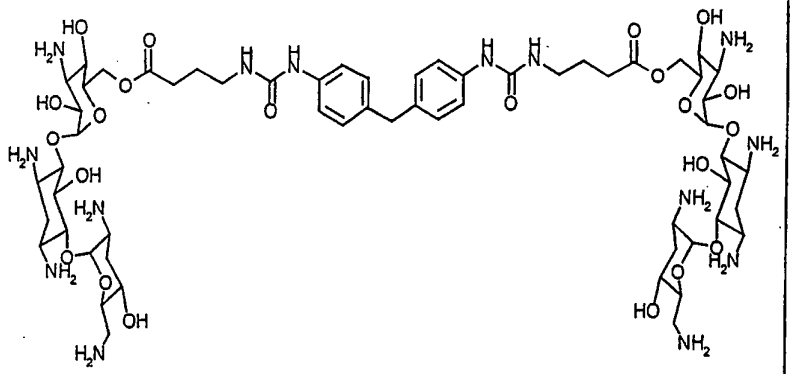
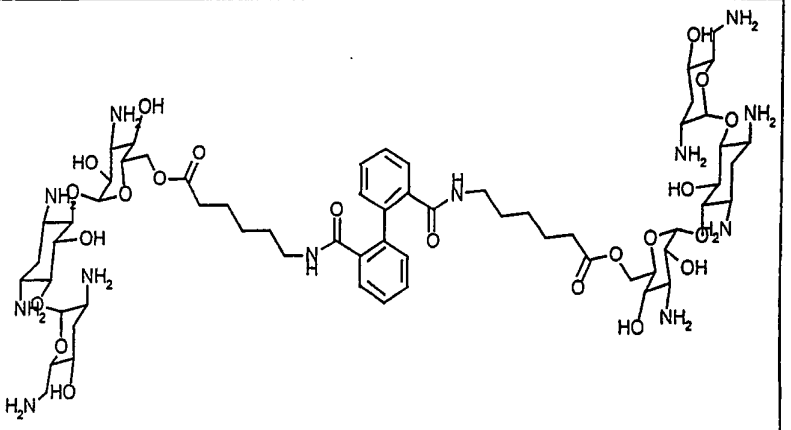
Compound	Modification reagent	Amino-glycoside Intermediate	General Method	Compound Structure
35	4,4'-Methylenebis(phenylisocyanate)	**5**	D	
36	Diphenic acid	**6**	C	

Table 4 cont: Synthetic & Structural Details for Dimeric Aminoglycosides

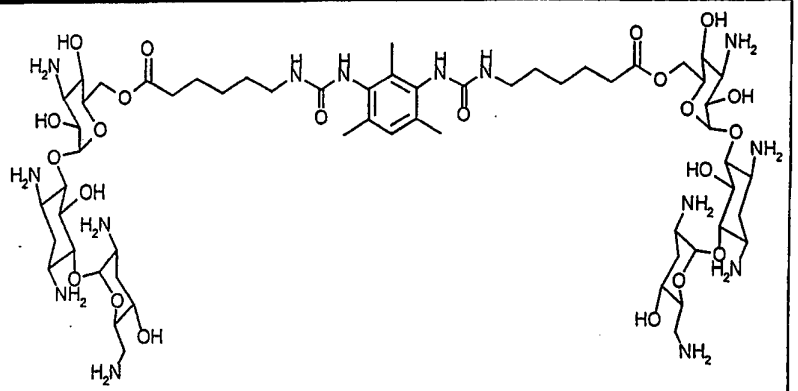
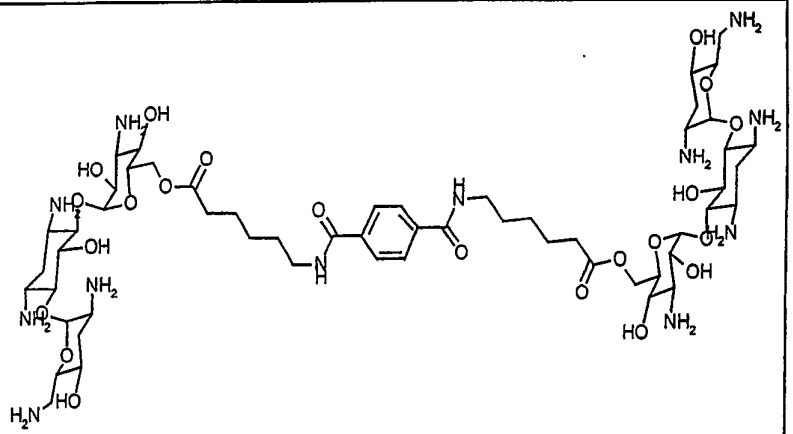
Compound	Modification reagent	Amino-glycoside Intermediate	General Method	Compound Structure
37	2,4,6-Trimethyl-1,3-phenylenediisocyanate	**6**	D	
38	Terephthalic acid	**6**	C	

Table 4 cont: Synthetic & Structural Details for Dimeric Aminoglycosides

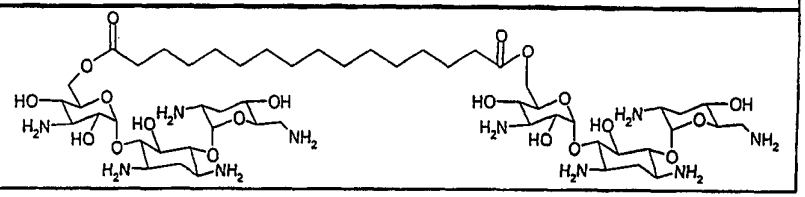
Compound	Modification reagent	Amino-glycoside Intermediate	General Method	Compound Structure
39	Cesium 1,16-hexadecanedioate	**2**	A	

Table 5: Characterization data for compounds 1 - 20

Compound ID	Calculated MW	Observed m/z	HPLC Retention time (min)	GRADIENT/HPLC Method Initial %B-Final %B_Duration
1	735.92	736.2$[M+H]^+$	2.32	GREEN; 5-100_1.1mins
2	627.70	628.1$[M+H]^+$	24.4	BLUE: 5-40_28mins
3	677.75	678$[M+H]^+$	26.5	BLUE; 5-40_28min
4	585.66	586.14$[M+H]^+$	21	BLUE; 5-40_28min
5	663.73	664$[M+H]^+$	26.25	BLUE; 5-40_28min
6	597.67	598$[M+1H]^{1+}$	22.75	BLUE; 5-40_28min
7	571.63	572.05$[M+1H]^{1+}$	20.05	BLUE; 5-30_28min
8	568.63	569.1$[M+1H]^{1+}$	2.65	GREEN; 5-100_1.1mins
9	601.66	602$[M+1H]^{1+}$	21.5	BLUE; 5-40_28mins
10	613.71	614$[M+1H]^{1+}$	28.75	BLUE; 5-20_28mins
11	655.63	655.9$[M+1H]^{1+}$	28.5	BLUE; 5-20_28mins
12	603.65	604$[M+1H]^{1+}$	20.5	BLUE: 10-30_28mins
13	615.68	616$[M+1H]^{1+}$	18.75	BLUE: 10-40_28mins
14	713.87	714$[M+1H]^{1+}$	31	BLUE; 10-40_28mins
15	572.62	572.9$[M+1H]^{1+}$	21	BLUE; 0-20%_28mins
16	612.68	613.03$[M+1H]^{1+}$	11.2	BLUE; 5-40_28mins
17	610.67	611.13$[M+1H]^{1+}$; 1221.25$[2M+H]^{1+}$	20.3	BLUE; 5-30_28mins
18	611.65	611.9$[M+1H]^{1+}$	15.5	BLUE; 5-15_28mins
19	610.67	611.05$[M+1H]^{1+}$; 1221.28$[2M+H]^{1+}$	26.5	BLUE; 5-20_28mins
20	587.63	587.95$[M+1H]^{1+}$; 1174.3$[2M+H]^{1+}$	23	BLUE; 5-30_28mins

Table 5 cont: Characterization data for compounds 21 - 46

Compound ID	Calculated MW	Observed m/z	HPLC Retention time (min)	GRADIENT/HPLC Method Initial %B-Final %B_Duration
21	580.68	581.1$[M+1H]^{1+}$	10.75	BLUE; 0-20_28mins
22	728.80	729.1$[M+1H]^{1+}$	22	BLUE; 10-20_28mins
23	537.61	538$[M+H]^{+}$;	0.92	GREEN; 5-100_1.1mins
24	720	720.2$[M+1H]^{1+}$	23.3	BLUE; 5-80_28min
25	705.94	706.2$[M+H]^{+}$	2.43	GREEN; 5-100_1.1mins
29	944	945$[M+H]^{+}$; 472.5$[M+2H]^{2+}$	NA	NA
30	661.80	662.66$[M+H]^{+}$	29.54	RED; 20-50_20mins
31	916	916.74$[M+H]^{+}$	30.5	BLUE; 15-50_28min
32	583	583.05$[M+H]^{+}$	9.7	BLUE; 0-10_28min
33	583	583.11$[M+H]^{+}$	0.57	GREEN; 5-100_1.1mins
34	1265.39	1265.3$[M+1H]^{1+}$; 633.4$[M+2H]^{2+}$	13	BLUE; 10-15_28mins
35	1355.52	1355.2$[M+1H]^{1+}$; 678.3$[M+2H]^{2+}$	23	BLUE; 10-25_28mins
36	1367.57	1367.3$[M+1H]^{1+}$; 684.5$[M+2H]^{2+}$	16.5	BLUE; 10-40_28mins
37	1363.58	1363.3$[M+1H]^{1+}$; 682.5$[M+2H]^{2+}$	21.25	BLUE; 5-30_28mins
38	1291.47	1291.3$[M+1H]^{1+}$; 646.5$[M+2H]^{2+}$	16.5	BLUE; 0-30_28mins
39	1185.43	1185.5$[M+1H]^{1+}$; 593.4$[M+2H]^{2+}$	19.2	BLUE; 5-80_28mins
40	640	639.99 $[M+1H]^{1+}$ 641.94 $[M+1H]^{1+}$	ND	-
41	606	607.09 $[M+1H]^{1+}$	ND	-
42	575	576.08 $[M+1H]^{1+}$	ND	-
43	578	579.11 $[M+1H]^{1+}$	ND	-
44	577	578.15 $[M+1H]^{1+}$	ND	-
45	577	578.09 $[M+1H]^{1+}$	ND	-
46	570	570.97 $[M+1H]^{1+}$	23	BLUE; 5-25_28mins

The structural characterisation of compounds 26, 29 and 30 by MS/MS using a Finnigan ion trap mass spectrometer is shown in Figures 2 to 4.

Preparative RP-HPLC Conditions

	Method GREEN		Method RED			Method BLUE	
Column support and dimensions	Phenomenex 3µC8 SiO_2 (20mm x 4mm)		Phenomenex 10µC8 SiO_2 (25cm x 2.12cm)			Phenomenex 10µC8 SiO_2 (25cm x 2.12cm)	
Flowrate	1ml/min		10ml/min			10ml/min	
Solvent A	0.1%TFA/H_2O		0.1%TFA/H_2O			0.1%TFA/H_2O	
Solvent B	0.06%TFA/CH_3CN		0.06%TFA/CH_3CN			0.06%TFA/CH_3CN	
Gradient Method	Time (Min)	A(%)	B(%)	Time (Min)	B(%)	Time (Min)	B(%)
	0	95	5	0	Initial*	0	Initial*
	0.5	95	5	15	Initial*	2	Initial*
	1.60	0	100	35	Final*	30	Final
	2.10	0	100	39	100	40	100
	2.30	95	5	42	100	45	100
	5	95	5	45	Initial*	47	Initial*

*For gradient information refer to **Table 5.**

Example 6: Antibiotic activities and *in vitro* translation inhibition of aminoglycoside prodrugs

Antibiotic activities were determined using measurement of minimum inhibitory concentrations by standard procedures referring to the Manual of Commercial Methods in Clinical Microbiology. Ed. Allan L.Truant. ASM Press (2002) and are reported as concentrations (μM).

	MIC *P.aeruginosa* ATCC27853 (μM)	MIC *E.coli* ATCC25922 (μM)
Tobramycin	2	-
Amikacin	7.5	-
Neomycin	30	-
Kanamycin A	30	-
1	8	16
2	8	-
3	>64	-
4	16	-
5	32	-
6	64	-
7	64	-
8	8	-
9	>64	-
10	>64	-
11	32	-
12	4	-
13	2	-
14	>32	>32
15	4	4
16	16	16
17	16	>32
18	16	16
19	32	>32
20	16	16
21	8	16
22	32	32
23	32	-
24	16	-
25	16	-
29	64	-
30	64	-
31	32	-
32	>64	-
33	>64	>32
34	8	8
35	4	8
36	8	8
37	16	16
38	32	16
39	4	-

Example 7: Assessment of enhanced residency time of aminoglycoside prodrugs

Rodents were anaesthetized with Ketamine/Domitor mixture according to standard procedures[6] and dosed by the intra-nasal route with a solution containing an equimolar quantity of the compound of interest and kanamycinA (as internal standard) at a dose volume of approximately 3.0ml/kg. The rodent was held in the vertical position during dosing of 30µL per nostril. At 168 hours post-dose, mouse lungs were harvested and levels of compound in the lung tissue were assessed by analytical methods. Any analytical method suitable for detection of this type of compound may be used[7].

Since prodrugs were designed to increase aminoglycoside concentrations over extended periods of time, the data is expressed as the ratio of tobramycin to kanamycinA present 168 hours post dose. The data clearly indicates that for the homologous series of compounds 23, 25, 27 and 28, levels of tobramycin relative to kanamycinA are influenced by the nature of the prodrug component as shown in Fig. 1.

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It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.